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Stability of Dexamethasone Formulations Marketed in Sudan

By

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Contents

List of Abbreviations

GMP	Good manufacturing practice.
GSP	Good storage practice.
GDP	Good distribution practice.
C°	Degrees centigrade.
TLC	Thin layer chromatography.
UV	Ultraviolet.
IR	Infrared.
UV-VIS	Ultraviolet-Visible.
nm	Nanometer.
HPLC	High performance liquid chromatography.
µl	Micro litter.
CDS	Central drug stores.
RH	Relative humidity.
USP	United state pharmacopoeia.
ml	Milliltrese.
mm	Millimeters.
RSD	Relative standard deviations.
pH	Hydrogen number.
rpm	Rotation per minute.
R^2	Correlation coefficient.
K	Rate constant.
Ca	concentration of component a.
Cb	concentration of component b.

t_{10}	Time needed to lose 10% of concentration.
t_{50}	Time needed to reach 50% of concentration.
FIFO	First in first out.
FEFO	First expired first out.
Bp	British pharmacopoeia.
FDA	Food and drug administration.

Abstract

Objectives

Many complaints have been made by Sudanese doctors about the lack of effectiveness of some common medicinal products in spite of their labeled manufacturer's shelf life is still valid. So, the effect of the real storage conditions in Sudan on stability of the different dosage forms of drugs circulated in Sudan market is needed.

Dexamethasone was the drug targeted in this study. It is a life saving drug use as a potent anti-inflammatory and anti-allergic.In common with all glucocorticoid their suppressive action on the hypothalamic-pituitary-adrenal axis is greatest and most prolonged when they are given at night. In most individuals a single dose of 1 mg of Dexamethasone at night, is sufficient to inhibit corticotropin secretion for 24 hours. This is the basis of the 'overnight dexamethasone suppression test' for diagnosing Cushing's syndrome. Dexamethasone is also appropriate for conditions where water retention would be a disadvantage. A corticosteroid may be used in the management of raised intracranial pressure or cerebral oedema that occurs as a result of malignancy; a high dose of dexamethasone is generally used. However, a corticosteroid should not be used for the management of head injury or stroke because it is unlikely to be of benefit and may even be harmful.It is found in different dosage forms in Sudan (tablets, injections, oral solutions and ointments) which are stable in normal controlled room temperature but when they were exposed to drastic conditions they may show changes due to instability.

Methodology

Samples were collected directly from local agent and the central medicine stores (C D S). Samples were analyzed first at zero time, then three parallel lines for distribution were done:-

1- One batch of each drug was stored in stability cabinet (so as to cover the analysis needs) at temperature of 30 ºC ± 2 and 70 % ± 5 R H (Registration requirement).

2- The same batch was distributed to different areas in Sudan. These areas (Dongola, Port Sudan, Elfashir, Elgadarif and Khartoum) were selected so as to cover the different climatic conditions in Sudan. The distribution was done using controlled temperature vehicles and then stored at actual storage conditions found in local pharmacies. Samples were collected at the time of test and send by controlled temperature vehicles as follows:-

- After 4 month of storage.
- After 8month of storage.
- After12 month of storage.

3. The same batch was stored in well controlled conditions (local agents drug stores).

Samples were analyzed using the USP (30) methods. The results obtained from stability cabinet were compared with those obtained from the different areas of Sudan and those obtained from the controlled conditions.

Results:-

As show in table No (1) :-

(1) Dexamethasone sodium phosphate injection:-

The assay results obtained after follow up for one year from different areas in Sudan showed changes between 20.0% - 25.0%, while samples stored at C.D.S. showed about 3.2% changes.

The pH changes were between 0.30 - 0.70.

(2) Dexamethasone tablets :-

The assay results obtained after follow up for one year from different areas in Sudan showed changes between 6.8 % -14.8 % while samples obtained from another company showed about 13.8% - 13.9% changes in both company stores and pharmacy through 6 month.

The dissolution test results showed an increase in U.V. reading after 4 month due to the degradation product which absorb at the same wavelength .

(3) Dexamethasone ointments :-

The assay results obtained after follow up for one year from different areas in Sudan showed between 1.0% - 12.5% changes .

(4) Dexamethasone oral solution :-

The assay results obtained after follow up for one year from different areas in Sudan showed between 34.6 %- 37.3% changes, while samples stored at company store showed about 5.7% changes.

The pH changes were between 0.3 - 0.4

Conclusion

The different dosage forms of dexamethasone (tablets, ointments, oral solutions and injections) were clearly affected when exposed to the real storage conditions in the market comparing with those stored in controlled storage conditions. This indicate that the storage conditions in the market is not suitable for these dosage forms to remain stable over its shelf life.

مستخلص الاطروحه

الأهداف:

نتيجة للشكاوى المتكررة من الأطباء فيما يخص فعالية الأدوية بالرغم من أنها لا تزال صالحة للاستعمال حسب ما هو موضح على الديباجة كان لا بد من دراسة تأثير ظروف التخزين الحقيقية التي يتعرض لها الدواء في السودان على ثباتية الأدوية بأشكالها الصيدلانية المختلفة. لقد تم اختيار عقار الدكساميثازون في هذه الدراسة ممثلا فى اشكاله الصيدلانية المختلفة الموجودة في السوق المحلى مثل الاقرص، المراهم ,الحقن والشرابات و ذلك لأنه عقار منقذ للحياة و يستخدم في علاج حالات كثيرة كمضاد للالتهابات ومضاد للحساسية. من المعروف أن الدكساميثازون ثابت في ظروف التخزين الاعتيادية المنصوص عليها في الديباجة بينما يظهر تغيرات تدل على عدم الثبات عند تعرضه لظروف تخزين دون المواصفات ونسبه لتباين ظروف المناخ من منطقه لأخرى في السودان مما قد يؤثر على ثبات الدواء كان لا بد من إجراء هذه الدراسة.

الطريقة:

تم تجميع العينات من مصادرها (الوكيل بالسودان أو الإمدادات الطبية) و من تم توزيعها عبر ثلاثة قنوات :-

1- وضعت العينات في حضانة مخصصة لإجراء دراسات الثبات فى درجة حرارة ($30 \pm 2\ C^{\circ}$) ورطوبة نسبية ($70\% \pm 5$) ثم تم تحليلها وفق طرق التحليل الواردة في دستور الأدوية الامريكى (30).

2- وزعت نفس التشغيلات على مناطق ذات مناحات متباينة شملت كل من

الخرطوم، دنقلا، القضارف، بور تسودان والفاشر بحيث تم تخزينها داخل الصيدليات العامة بتلك المناطق بحيث تتعرض لظروف التخزين الحقيقية وبالتالي نتمكن من معرفه تأثيرها على ثبات و فعالية الأدوية وقد تم تحليلها كما يلى :-

بعد 4 أشهرمن التخزين.

بعد 8 أشهر من التخزين.

بعد 12 شهر من التخزين.

3- نفس التشغيلات تم تخزينها في مخازن يوجد بها تحكم في درجات الحرارة والرطوبة وقد تم تحليلها بعد 12 شهر.

النتائج

الحقن

أظهرت نتائج التحليل لعينات الدكساميثازون من المناطق المختلفة عند متابعتها لمدة عام أن نسبة التغير تتراوح بين 20.0% - 25.0%.بينما نسبة التغيرفى العينات المحفوظة في مخازن الإمدادات هي 3.2%. التغير في درجة الأس الهيدروجيني يتراوح بين 0.7 -0.3

الأقراص

أظهرت نتائج التحليل لعينات الدكساميثازون من المناطق المختلفة عند متابعتها لمدة عام أن نسبة التغير تتراوح بين % 14.8 - 6.8% بينما نسبة التغيرفى العينات الماخوذه من شركه أخرى هي 13.9%-13.8%خلال ستة أشهر.

أما فيما يخص نتائج اختبار الانحلالية فقد أظهرت زيادة كبيرة في القراءات بعد أربعه أشهرنتيجه لان ناتج تفكك الدواء يعطى قراءة في ذات الموجه.

المراهم

أظهرت نتائج التحليل لعينات الدكساميثازون من المناطق المختلفة عند متابعتها لمدة عام أن نسبة التغير تتراوح بين. % 12.5 - 1.0%.

الشرابات:

أظهرت نتائج التحليل لعينات الدكساميثازون من المناطق المختلفة عند متابعتها لمدة عام أن نسبة التغير تتراوح % 34.6 - 37.3%. بينما نسبة التغير فى العينات المحفوظة في مخازن الشركة 5.7% التغير في درجة الأس الهيدروجيني يتراوح بين 0.4 -0.3

الخلاصة:

أظهرت الدراسة تدهورا واضحا في محتوى المادة الفاعلة في الأشكال الصيدلانية المختلفة من الدكساميثاذون المخزنة في ظروف التخزين الحقيقية في مناطق السودان المختلفة عند مقارنتها بتلك المحفوظة في ظروف تخزين محدده مما يدل على أن ظروف التخزين المتاحة في الصيدليات غير ملائمة للحفاظ على ثبات الادويه خلال فترة صلاحيتها.

List of tables

List of figure

Chapter 1
Introduction

1. 1.Definitions:-

1.1.1. Drug product:-

The dosage form in the final immediate packing intended for marketing.

1.1. 2.Excipients:-

Any thing other than the drug substance in the dosage form.

1.1.3. Active substance:-

The unformulated drug substance which may be subsequently formulated with excipients to produce drug substance.

1.1.4. Dosage forms:-

A pharmaceutical product type that contains a drug ingredient generally, but not necessarily in association with excipients.

1.1.5. Expiry date:-

Date placed in the container/labels of drug product designating the time during which a batch of the product is expected to maintain within specifications.

1.1.6. Storage condition tolerance:-

The acceptable variation in temperature and relative humidity of storage facilities (± 2C˚ for temperature and ± 5% for humidity).

(Jens T. Carstensen second edition).

1.1.7. Tablets:-

Tablets are solid preparations each containing a single dose of one or more active substances and usually obtained by compressing uniform volumes of particles. Tablets are intended for oral administration .Some are swallowed whole, some after being chewed ,some are dissolved or dispersed in water before being administered and some are retained in mouth where the active substance liberated.

The particles consist of one or more active substance with or without exipients such as diluents, binders, disintegrating agents, glidants, lubricants, substances capable of modifying behavior of preparation in digestive tract ,coloring matter authorized by the competent authority and flavoring substances.

Tablets are usually right, circular solid cylinders, the end surface of which are flat or convex and edges of which may be bevelled .They may have break-marks or may bear a symbol or other markings. Tablets may be coated.

1.1.8. Injections:-

Parental preparations are defined as solutions, emulsions in water for injections, and does not preclude the inclusion of suitable excipients where necessary. In particular, aqueous parental preparation for administration by subcutaneous, intradermal, intramuscular, or in case of larger volumes, intravenous rout, should, if possible, be made isotonic with blood by addition of sodium chloride or a suitable substances .However if buffering agents are used in preparations intended for intraocular or intracardiac injection or in preparations that may gain access to cerebrospinal fluid, great care should be taken to ensure that the nature and concentration of chosen agent are suitable for the intended rout of administration . Where the active ingredient is susceptible to oxidative degradation appropriate precautions should be taken.

1.1.9. Oral liquids:-

Liquid preparations for oral use are usually solutions, emulsions or suspensions containing one or more active substances in suitable vehicle, they may, however consist of liquid active substances used as such.

The vehicle for many preparations for oral use is chosen having regard to the nature of the active substance and to provide organoleptic characteristics appropriate to the intended use of the preparation.

Oral liquids may contain suitable antimicrobial preservatives, antioxidants and other excipients.

(The British pharmacopoeia commission, 2009)

1.10. Ointments:-

Ointments are semisolid preparations intended for external application to the skin or mucous membranes.

Ointments bases recognized for use as vehicle fall into four general classes: the hydrocarbon bases, the absorption bases, the water removable bases, and the water soluble bases .Each therapeutic ointment possesses as its base a representative of one of these four general classes.

(United states pharmacopoeia convention 30 NF)

1.2. Drug stability:-

A medicinal product is designed to possess certain desirable properties of which the following are of major importance:-

When the product is administered by the specified route, the active constituent should achieve the required rate and extent of bioavailability.

The product itself should be efficacious, safe and acceptable to the patient; it should be convenient in use and stable.

(Walter Lund 12 edition)

Stability of pharmaceutical product may be defined as the capability of particular formulation (in specific container/closure system) to remain within its physical, chemical, microbiological, therapeutic and toxicological specification.

(Remington pharmaceutical sciences 17th edition 1985)

There are many factors that affect the stability of pharmaceutical products, including the stability of active ingredients, the potential interaction between active and inactive ingredients, the manufacturing process, the dosage form, the container liner- closure system, the environmental conditions encountered during shipment, storage, handling and length of time between manufacture and usage.

(Remington pharmaceutical sciences 17th edition 1985)

The term stability with respect to the drug dosage form refers to the physical and chemical integrity of the dosage unit and when appropriate, the ability of dosage unit to maintain protection against microbiological contamination. The stability can be influenced by environmental conditions of storage (temperature, light, air and humidity).

(United states pharmacopoeia convention 24)

Stability is often expressed in quantitative terms as the shelf life: that is the time during which the medicinal product is predicted to remain fit for its intended use under specified condition of storage. The shelf life of medicinal product kept in its closed container under specified conditions is commonly defined as the time from manufacturing or preparation until the original potency or content of active constituents has been reduced by 10%.This time is known as ($t_{10\%}$) . This 10% limit of chemical degradation is usually considered to be acceptable in practice but more stringent limits may need to be imposed if degradation products are more toxic or irritant than is the drug. Although it is often convenient to express shelf life solely in terms of chemical stability of active constituent, it is essential that the other desirable properties of the product are retained during storage.

(Walter Lund 12 edition)

1.2.1 Types of drug stability:-

1.2.1.1. Chemical stability.

1.2.1.2. Physical stability.

1.2.1.3. Microbiological stability.

1.2.1.1Chemical stability:-

Chemical degradation of the active constituent in a medicinal product often results in loss in potency .The degradation products of drugs may be very toxic (for example epianhydrotetracycline formed from tetracycline) so that clinical use of preparation may be unacceptable if the extent of decomposition is relatively great . Degradation of an excipient can pose problems of physical or microbiological stability (for example hydrolysis of sorbitan ester may result in sufficient loss in its stability to produce an interfacial film that a formulated emulsion may crack). Since chemical reaction proceed more readily in liquid state than in the solid state, then solid dosage forms are of greater stability than others .

(Walter Lund 12 edition)

1.2.1.1.1.Factors that may affect chemical stability :-

The rate of chemical reaction of a drug or excipient in a medicine may be affected by physicochemical factors such as :-

1.2.1.1.1.1.pH :-

In reversible oxidations the standard reduction potential may depend on pH; thus pH may affect the tendency of a drug to be oxidized.

Hydrolysis reactions are often catalyzed by both hydrogen ions and hydroxide ions. Since hydroxide usually exert a greater catalytic effect than hydrogen ions, the minimum degradation rate (the maximum stability) of many

drugs is in the range of pH 2 to 5 . In addition temperature may modify the e0ffects of pH.

The rate of other reactions such as isomerisation may be influenced by pH.

1.2.1.1.1.2.General acids and bases :-

In addition to catalysis by hydrogen ions and hydroxide ions (specific acid - base catalysis), certain hydrolysis reactions are catalyzed by other acidic and basic species

(General acid - base catalysis) such as those salts used to buffer solutions of drugs.

1.2.1.1.1.3.Ionic strength :-

Addition of an inert electrolyte to an aqueous solution of drug may exert a direct effect on stability even though there is no chemical interaction between the drug and the electrolyte (primary salt effect) which depend on the concentration of the added salt and on the charges born by the reacting ions. If ions bear the same charge, the addition of salt increases the rate of degradation. In contrast, when ions are of opposite charge, the rate is decreased. When one of the reactant is not charged, addition of a salt should not affect stability of the drug.

Electrolytes may also indirectly influence the rate of reaction by modifying the ionization constant of week acids or bases present in buffer solutions and enhancing or reducing catalysis by general acids and bases (secondary salt effect).

1.2.1.1.1.4.Nature of solvent :-

The influence of the solvent on the rate of degradation of a drug depends largely on the dielectric constant of solvent and on the electrical charge on the drug .When the charge on the drug and attacking ion are the same , part or

complete replacement of water(high dielectric constant) by alcohol ,propylene glycol ,or glycerol(lower dielectric constants) usually lowers the rate of degradation . Where the charge are opposite reduction in dielectric constant of solvent would be expected to increase the rate of degradation.

(Walter Lund 12 edition)

1.2.1.1.1.5.Drug concentration :-

The effects of drug concentration on degradation depend on the order of reaction. For example, if the reaction follows first – order kinetics, the rate is directly proportional to drug concentration but the time for certain proportion to decompose is independent of concentration.

Where the mechanism of degradation and order reaction depend on drug concentration, the kinetic are complex. In dilute solution, ampicillin degrades by pseudo- first order kinetics but in more concentrated solutions the reaction becomes third order, concentrated solution of this drug degrade more rapidly than dilute solutions. .

(Walter Lund 12 edition)

For degradation of drugs where the molecules associate to form micelles ,the order of reaction may depend on drug concentration ;for example the thermal degradation in dark of promethazine hydrochloride in aqueous solution appeared to follow first order kinetics at concentrations below 0.5% but zero order kinetics at concentrations above 3.0%

(Meakin B J ,etal, 1978)

1.2.1.1.1.6.Surfactants :-

Surfactants are used as excipients in the formulations of medicines, especially as dispersing emulsifying or solubilising agents. The effects of surfactants on the stability of drugs may depend on factors such as concentration, solubility, chemical nature, and chain length of the surfactant, the mechanism of degradation of the drug, its site of solubilisation, and the charge on the attacking ion.

The auto-oxidations of the oil methyl linoleate in simple dispersion in water was very slow because very little oil was soluble; addition of potassium laurate results in formation of an emulsion in which part of the methyl lineate was solubilised in micelles of the surfactant and part was present as oil droplets. Autoxidations of the emulsion was rapid since the micelles provided especially favorable conditions for initiation of the reaction; the free radicals formed then migrated from the micelles into the water and diffused into oil droplets where propagation proceeded rapidly. Further addition of the surfactant resulted in more oil molecules becoming solubilised until the solubilisation process was complete; at this stage, the rate of auto-oxidation was much lower because there were no oil droplets present to enable rapid propagation to occur ; at high concentrations of the surfactant the auto-oxidation rate was further reduced; this effect was attributed to a smaller number of methyl linoleate in each micelle.
(Carless JE and Nixion JR.J 1960)

1.2.1.1.1.7.Peroxides :-

The presence of highly labile peroxides may catalyze the initiation and propagation stages of auto-oxidation since these substances easily form free radicals. Peroxides are commonly formed in fixed oils and in diethyl ether.

(Walter Lund 12 edition)

1.2.1.1.1.8.Heavy metal ions :-

Traces of heavy metals ions often catalyze auto-oxidation reaction. The mechanism of action of heavy metals is demonstrated by shortening of the lag phase. The mechanism of action of heavy metals ions appears to be associated with the rapid formation of free radicals and is thought to be due to the ability of such cations to change readily from one valency state to another: for example, cupric to cuprous. Sources of heavy metal ions include metal manufacturing equipment, water, drugs of natural origin excipients, and chromic acid used for cleaning of glassware.

1.2.1.1.1.9.Oxygen:-

Many oxidation reactions that occur in the ingredients of medicines are autoxidations where oxygen is necessary for propagation of the chain reaction. Often a low concentration of oxygen in the product is sufficient to permit considerable oxidation to take place. The partial pressure of oxygen in the air in container may be important.

1.2.1.1.1.10.Carbon dioxide :-

The partial pressure of gaseous carbon dioxide in the container or its concentration in the product may affect the rate and extent of reactions that involve the uptake of this gas.

1.2.1.1.1.11.Water :-

Drug substances, excipients, and solid dosage forms such as tablets and capsules may contain small amount of water .Since substances and solid dosage forms are sometimes kept at a relatively high humidity they may sorbs water on the surface which may lead to dissolution of the drug and to degradation.

(Walter Lund 12 edition)

In a study of aspirin stability in solid state the degradation depended on water vapor pressure .The mechanism is that water is rapidly sorbed as a monolayer or multilayer on the surface of the drug particles ; The amount sorbed is a function of water vapor pressure . Some of aspirin then dissolves in the sorbed water to form a saturated solution; as aspirin in solution hydrolyses to acetic and salicylic acid ,more of the solid aspirin dissolves so that the solution remains saturated .

(**Leeson** LJ ,and Maddocks AM. 1958).

1.2.1.1.1.12.Temperature :-

For many reactions an increase in temperature enhances the rate constant (Arrhenius equation).Information on the effect of temperature is important in the prediction of shelf life of products marketed in the tropics .The effect of temperature on some oxidation reactions may operate in opposing directions, although a rise in temperature results in a higher degradation rate, it also decrease the solubility of oxygen in water so affects the rate of oxidation (Do not follow the Arrhenius equation).

Storage at low temperatures may adversely affect the stability of some medicines for example: polymerization of formaldehyde in aqueous solution proceeds more rapidly below 15˚. **(**Walter Lund 12 edition)

1.2.1.1.1.13.Light :-

Photochemical reactions involve the absorption of light of particular wave length in the ultraviolet or visible regions of the spectrum. The radiant energy must exceed a threshold before the reaction can occur .However not all absorbed light leads to reaction, since some may be converted to heat or transferred to molecules of another substance, or emitted at the same or different wave length .As the wave length increases there is a decrease in radiant energy.

1.2.1.1.1.14.Ionizing Radiation :-

The use of ionizing radiation to sterilize medicinal products may result in decomposition. At the radiation dose (25 KGY) commonly recommended for sterilization, the nature and extend of degradation of many substances is unacceptable.

1.2.1.1.1.15.Mechanical processes :-

Grinding can cause drug solvates or hydrates to become more chemically unstable since it weaken the bonding force between drug molecule and its water of crystallization; thus liberated water molecules can take part in hydrolysis reaction.

(Walter Lund 12 edition)

1.2.1.2.2.Reactions that cause chemical degradation:-

A variety of chemical reactions can result in the degradation of drug substances and excipients; sometimes more than one reaction may occur at the same time .These reactions are:-

1.2.1.1.1.1.Hydrolysis :-

For most parenteral products, the drug comes into contact with water and, even in solid dosage forms; moisture is often present, albeit in low amounts. Accordingly,

hydrolysis is one of the most common reactions seen with pharmaceuticals. Hydrolysis is often the main degradation pathway for drug substances having ester and amide functional groups within their structure. It involves the reactions of molecules with water that results in cleavage.

Enzyme catalyzed hydrolysis may take place in drugs of natural origin.

*(*Sumie Yoshioka and Valentino J. Stella)

1.2.1.1.1.2.Dehydration :-

Dehydration reaction occurs occasionally in drugs.

1.2.1.1.1.3.Oxidation :-

Oxidation is applied to reactions in which either one or more electropositive atoms, radicals or electrons are lost or more electronegative atoms or radicals are gained .Many drugs are prone to degrade by oxidation. Oxidation reactions are either redox reactions without addition of oxygen (reversible) or chain reaction in which a substance becomes slowly oxidized in the presence of atmospheric oxygen.

(Walter Lund 12 edition*)*.

1.2.1.1.1.4. Isomerisation :-

Isomerisation is the conversion of a substance into its geometric or optical isomers; these have the same structural formula but differ in astrochemical configuration.

1.2.1.1.1.4.1. Geometric Isomerisation: - This conversion involves changes in the relative spatial configuration of atoms or groups around ethylene double bonds or cyclic compounds .Different geometric isomers may possess different potencies.

1.2.1.1.1.4.2. Optical Isomerisation: - It is change in the optical rotation of substance as a result of presence of one or more chiral centers. Two types of optical isomerisation can be distinguished**:** Racemisation and Epimerization**.**

1.2.1.1.1.4.3. Racemisation:-

Racemisation involves the conversion of an optically active drug with one chiral center into an isomer whose structure is a mirror image of the original molecule (Enantiomers). The reaction continues until the concentration of the two enantiomers are equa. At this stage the solution of the resultant racemic mixture no longer rotates the plane of polarized light . Racemisation of some drugs may lead to more complex pharmacological or toxicological effects for example: enantiomers may differ in their affinity for receptors or may have opposite effects.

1.2.1.1.1.4.4. Epimerization:-

Occurs when there is more than one chiral center in which there is selective racemisation at one center; the equilibrium between the two epimers may not represent equal concentrations of each since the presence of other chiral centers may favour the formation of one epimer rather than the other so the optical rotation of the two isomers are not equal and opposite so the optical activity of the mixture of epimers at equilibrium will not be zero.

(Walter Lund 12 edition page)

1.2.1.1.1.5.polymerization :-

It is the combination of two or more identical molecules of substance to form more complex molecule, often polymerization follows a primary degradation process.

1.2.1.1.1.6.Photochemical reaction :-

When exposed to light many drugs and excipients are susceptible to degradation by a variety of photochemical reactions (oxidation, reduction).

In a photochemical reaction, the light-sensitive drug molecules may be affected directly or indirectly by light, depending upon how the absorbing photon

energy is transferred to the drug molecules. With a direct or indirect light-induced reaction, a drug can only undergo the photo-degradation process if the absorbed energy exceeds a threshold. Because ultraviolet radiation has higher energy, it is the main cause of many degradation reactions of light-sensitive drugs. Colored-glass containers are the most commonly used method to protect these types of drugs. Yellow-green glass gives the best protection in the ultraviolet region; amber glass also offers considerable protection from ultraviolet light, but little protection from infrared light.

The photochemical reaction is a very complex process; many variables may be involved in the photolytic degradation kinetics. The velocity of the photochemical reaction may be affected not only by the light source, intensity, and wavelength of the light, but also by the size, shape, composition, and color of the container. To properly determine the effects of light on the quality of a drug properly, standard light stability testing should consider all of the aforementioned variables. Once uniform standard light-stability testing procedures are instituted, proper packaging, storage environment, and expiration date for the light-sensitive drug can be established.

(Hanne Hjorth Tonnsen, 2004)

1.2.1.1.1.7.Radiation - induced reaction :-

Degradation by complex mechanisms due to exposure to ionizing radiation**.**

1.2.1.1.1.8.Decarboxylation :-

It is removal of carbon dioxide from substance .It may occur as primary reaction or as a secondary degradation reaction (for example procaine hydrochloride hydrolysis to form 4-amino benzoic acid which Decarboxylates to form aniline.

1.2.1.1.1.9.Absorption of carbon dioxide :-

Absorption of carbon dioxide lead to lower the pH and the free acid form is precipitated .As in solutions of calcium hydroxide.

(Walter Lund 12 edition*)*

1.2.1.3. Order of reaction:

It is the way in which concentration of reactant, or reactants, influences the rate of chemical reaction.

1.3.1. First order reaction:

The rate of a reaction is proportional to the first power of the concentration of a reactant and may be expressed mathematically as follow :

$- dc/dt = Kc$

When log c was plotted against (t) it will gave straight line; and the values of K, t_{10}, t_{50} was expressed as follow:

$K = 0.693/slop$

$t_{10} = 0.105/K$

$t_{50} = 0.693/K$

1.3.2. Second order reaction:

The rate of a reaction is proportional to concentration of each of two reactant or the second power of the concentration of one reactant and may be expressed mathematically as follow:

$- dc_A/dt = -dc_B/dt = K.c_A c_B$

t_{50} **OR** $t_{1/2} = 1/K a$

1.3.3. Zero order reaction:

The rate is independent of concentration of reactants. In such cases the rate is expressed as:

$- dc/dt = K$

And t_{10}, t_{50} was expressed as follow:

$t_{10} = 0.1a/K$

$t_{50} = 0.5a/K$

a = initial concentration

(Remington's 1985.)

1.2.1.2.Physical Stability :-

Physical changes may result in diminished bioavailability and efficacy or may adversely affect other properties such as dispersability, acceptability to the patient and convenience for use.

1.2.1.2.1.Types of physical changes:-

1.2.1.2.1.1.Volatility of constituents :-

Drugs, solvents and excipients with high vapor pressure can be lost from medicinal products during manufacture and storage .Such drugs should be kept in well-closed airtight containers to avoid loss of active constituents, also the degradation product in tablet may sublime and appear as a deposit on tablet surface or in the walls of the container (asprin → saliciylic acid) **.** Also volatility of solvents in solutions lead to potentially hazardous concentrations of preparations especially in poorly closed, partially- filled containers kept in a warm dry place. Also loss of water by evaporation from aqueous solutions in warm dry conditions may result in crystallization of the drug or excipients;

similarly creams may lose water and form thick layer on the surface; the emulsion may crack.

1.2.1.2.1.2.Changes in the water content of solids:-

Water can be sorbed on many solid drugs, exipients and medicinal products. In many substances water is bound rather than unbound. Bound water interacts physically with solids and its equilibrium vapor pressure is less than that of unbound water; bound water may be entrapped in pores or may be molecularly bound to form crystalline hydrates.

Many materials are hygroscopic (for example glycerol), that they gain (or loss) water in accordance with relative humidity; some are deliquescent that is they take up water and dissolve. Some salts (for example sodium sulphate) are efflorescent, their tendency to loss water to form lower hydrate or the anhydrous salt depend on relative humidity and temperature.

Storage of hard gelatin capsules at high relative humidity may result in the capsules becoming sticky and distorted.In very dry conditions water is desorbed from the capsules shells, causing shrinking.

In filled capsules , moisture may be transferred from the shell to its contents; if drug or excipients, are hygroscopic which could lead to chemical degradation , formation of hydrates ,or the production of a hard cement –like mass from which the drug would be slowly released after administration which lead to reduced bioavailability .

Soft gelatin capsules are specially sensitive to conditions of relative humidity greater than 60% since irreversible softening may occur, and they may become tacky and bloated.

The uptake or loss of water from solid dosage forms can result in undesirable changes in properties such as hardness, disintegration time, dissolution rate, bioavailability and efficacy.

Dry powders and granules may become aggregated to form a cake.

(Walter Lund 12 edition)

1.2.1.2.1.3.Sorption :-

Change in crystal form, habit, and size of solid can affect not only the physical properties of medicine but also its bioavailability.

1.2.1.2.1.4.Crystal form :-

Some drugs can exist in several crystal forms or polymorphs, which represent different arrangements of molecules with in the crystal lattice; although chemically identical polymorphs differ in physical properties because of differences in free energy.

At a given temperature and in a particular solvent, only one form is thermodynamically stable; other metastable forms tend to be converted to the stable form.

Metastable forms have a higher free energies, higher solubilities and dissolution rates and lower melting points than have the thermodynamically stable forms.

Conversion of a metastable form to the stable form of drug usually occurs more rapidly in suspension rather than in solid dosage forms and is often associated with crystal growth, which may lead to caking of the suspended particles.

Large fluctuations in storage temperatures should be avoided to minimize change in crystal form of a drug.

Polymorphic changes can also occur during mechanical treatment of solid drugs.

1.2.1.2.1.5.Crystal habit :-

Some crystal forms of substances may exist in different habits or external shapes; examples are prismatic, tabular, and isometric habits of an orthorhombic crystal form. Change in habit in a suspension can affect physical properties such as dissolution rate and the ability to flow through a syringe needle.

Large fluctuations in storage temperatures should be avoided to minimize change in crystal habit. .

1.2.1.2.1.6.Crystal growth :-

Crystals of a drug may grow in suspension because of temperatures fluctuation; dissolution of small crystals occurs when the temperature is raised followed by deposition into large crystals during cooling. Even at constant temperature crystal growth may occur in suspensions of drugs such as corticosteroids that contain very small particles, because such tiny crystals have a higher solubility in water but deposit on the larger crystals.

Crystal growth can be minimized by:-

Addition of surfactant or polymers.

Use a narrow size range of particles.

Increasing the viscosity of vehicle.

Avoiding large fluctuations in storage temperatures.

(**Walter Lund** 12 edition page 296)

1.2.1.2.1.7.Crystallization from solution :-

Crystals of a drug may be deposited from solutions because of a fall in temperature or change in pH in supersaturated solutions.

1.2.1.2.1.8 Precipitation in Galenicals :-

Inert matter derived from the contents of plant cells may coagulate in galenicals such as liquid extracts (tinctures); this precipitate is often known as ‹pitching ‹.

1.2.1.2.1.9.Physical change in Emulsions :-

The physical instability of emulsions occurs in three main forms: flocculation where the interfacial film is not broken;

(1) Cracking: - Where the globules coalesce because of rupture of the interfacial film.

(2) Creaming: - Where the globules move towards the surface of the product (emulsions).

(3) Sedimentation: - Where the movement of globules is down wards.

These changes are often accompanied by change in viscosity of the emulsion.

1.2.1.2.1.10.Physical change in suspensions :-

The physical rate change of a solid drug in suspension or in a liquid depends largely on the magnitude of the opposing forces of attraction and repulsion. Attraction between particles occurs because of relatively week London-van der Waal´s forces whereas repulsion is caused by electrical double layer that surrounds each particle. If the forces of repulsion are greater than those of attraction, the suspension is deflocculated, that is, each particle settles slowly and individually to form sediment; sometimes the particles may aggregate to form an indispensable cake. In contrast if the attractive forces predominate, the particles come together to form loose floccules that settle rapidly to produce a readily dispersed sediment; the suspension is said to be flocculated.

1.2.1.2.1.11.Other physical changes :-

Semisolids: - Ointments, creams and pastes may soften, harden, or become granular or gritty during storage Suppositories and pessaries may soften ,harden, or shrivel.

Uncoated tablets**:-** Tablets may crumble or break during packaging or transport ,crack or chips may be evident in tablet surfaces . Change in hardness, disintegration rate, or dissolution rate may occur. A moltteled appearance in coloured tablets may be caused during manufacture by intragranular migration of dye during wet granulation.

Coated tablets:-During manufacturer clumping of film coated tablets may occur where inadequate drying of the coat results in tacky tablets that stick to neighboring tablets.

1. 2.1.3 Microbiological stability :-

Many liquid medicines can support the growth of micro –organisms. Aqueous preparations such as solutions, suspensions, and oil in water in emulsions are especially susceptible to microbial growth .Growth may also occur in tablets and other solid dosage forms that contain some water .

Contamination of products with bacteria, moulds or yeasts may occur during manufacture, dispensing, storage, or use and may be derived from water and from drug substances or excipients ;other sources include packaging materials, premises, equipment, clothing, workers, and the atmosphere.

Proliferation of micro –organisms in medicinal products is unacceptable for the following reasons:-

The presence of pathogenic bacteria, moulds, yeasts, or endotoxines can be hazardous to the patient particularly in solutions or emulsions administered

by intravenous infusion or solutions introduced into the anterior chamber of the eye during surgical procedure.

(2) Contamination with non pathogenic micro organisms can result in spoilage of the product.

(Walter Lund 12 edition)

1.3. Storage and Distribution of pharmaceutical products:-

The quality of a pharmaceutical product can be affected by a lack of adequate control over numerous activities which occur during the distribution process. Furthermore the distribution process has generally not been well-emphasized with regard to the need for establishment, development, maintenance and control over the activities involved. . In order to maintain the original quality every activity in the distribution of pharmaceutical products should be carried out according to the principles of good manufacturing practice (GMP), good storage practice (GSP) and good distribution practice (GDP).

1.3. 1 Distribution

The division and movement of pharmaceutical products from the premises of the manufacturer of such products, or another central point, to the end user thereof, or to an intermediate point by means of various transport methods, via various storage and/or health establishments.

1.3.3.2.Good distribution practices (GDP) (new):-

Good Distribution Practices are that part of quality assurance that ensure that the quality of a pharmaceutical product is maintained through adequate control throughout the numerous activities which occur during the distribution process.

1.3.3.3. VEHICLES AND EQUIPMENT:-

- Vehicles and equipment used to distribute, store, or handle pharmaceutical products should be suitable for their use and appropriately protective of the products to prevent exposure to conditions that could affect their stability and packaging integrity, and prevent contamination of any kind.
- The design and use of vehicles and equipment must aim to minimize the risk of errors and permit effective cleaning and/or maintenance, in order to avoid contamination, build-up of dust or dirt and/or any adverse effect on the quality of pharmaceutical products being distributed.
- Dedicated vehicles and equipment should be used, where possible, when handling pharmaceutical products.
- Where non-dedicated vehicles and equipment are used, procedures must be in place to ensure that the quality of the pharmaceutical products will not be negatively influenced.
- Appropriate cleaning should be performed, checked and recorded
- Defective vehicles and equipment should not be used, and should either be removed or labeled as such.
- There should be procedures in place for the operation and maintenance of all vehicles and equipment involved in the distribution process, including cleaning and safety precautions.
- Vehicles, containers and equipment should be kept clean and dry and free from accumulated waste. A written cleaning programmed should be available, indicating the frequency of cleaning and the methods to be used.
- Vehicles, containers and equipment should be kept free from rodents, vermin, birds and other pests. There should also be written programmed

for such pest control. Cleaning and fumigation agents should not have an adverse effect on product quality.

- Equipment used for the cleaning of vehicles should be chosen and used so as not to constitute a source of contamination.
- Special attention should be given to the design, use, cleaning and maintenance of all equipment used for the handling of pharmaceutical products which are not in a protective shipping carton or case.
- Where special storage conditions (e.g. temperature and/or relative humidity), different from or limiting the expected environmental conditions, are required during transit these should be provided, checked, monitored and recorded. All monitoring records should be kept for a minimum of the shelf-life of the product distributed plus one year, or as required by national legislation. Recorded monitoring data should be reviewed on receipt of pharmaceutical products to assess whether required storage conditions have been met.
- Equipment used for monitoring conditions within vehicles and containers, e.g. temperature and humidity, should be calibrated.
- Vehicles and containers should be of sufficient capacity to allow orderly storage of the various categories of pharmaceutical products during transportation.
- Where possible mechanisms should be available to allow for the segregation during transit of rejected, recalled and returned pharmaceutical products as well as suspected to be counterfeits. Such goods must be securely packaged, clearly labeled, and be accompanied by appropriate supporting documentation.

- Measures should be in place to prevent unauthorized persons from entering and/or tampering with vehicles and/or equipment, as well as to prevent the theft or misappropriation thereof.

.**(WHO** Working document QAS/04.068/Rev.2)

1.3.2. Storage:-

The storing of pharmaceutical product up to the point of use under specified condition of temperature and relative humidity.

1.3.2.1. Premises, Warehousing, and Storage:-

Good storage practice (GSP) is applicable in all circumstances where pharmaceutical products are stored throughout the distribution process**.**

1.3.2.2. Storage areas:-

- Precautions must be taken to prevent unauthorized persons from entering storage areas.

  Storage areas should be of sufficient capacity to allow the orderly storage of the various categories products, namely bulk and finished products, products in quarantine, and released, rejected, returned or recalled products.
- Storage areas should be designed or adapted to ensure good storage conditions. In particular, they should be clean and dry and maintained within acceptable temperature limits.Where special storage conditions are required on the label (e.g. temperature, relative humidity), these should be provided, checked, monitored and recorded. Pharmaceutical products should be stored off the floor and suitably spaced to permit cleaning and inspection. Pallets should be kept in a good state of cleanliness and repair.
- Storage areas should be clean, and free from accumulated waste and vermin. A written sanitation program should be available indicating the

frequency of cleaning and the methods to be used to clean the premises and storage areas.

There should also be a written program for pest control. The pest-control agents used should be safe, and there should be no risk of contamination of the materials and pharmaceutical products. There should be appropriate procedures for the clean up of any spillage to ensure complete removal of any risk of contamination.

- Receiving and dispatch bays should protect products from the weather. Reception areas should be designed and equipped to allow containers of incoming pharmaceutical products to be cleaned, if necessary, before storage.
- Where quarantine status is ensured by storage in separate areas, these areas must be clearly marked and their access restricted to authorized personnel. Any system replacing physical quarantine should provide equivalent security. For example, computerized systems can be used, provided that they are validated to demonstrate security of access.
- If sampling is performed in the storage area, it should be conducted in such a way as to prevent contamination or cross-contamination. Adequate cleaning procedures should be in place for the sampling areas.
- Physical or other equivalent validated (e.g. electronic) segregation should be provided for the storage of rejected, expired, recalled or returned products. The products, and areas concerned should be appropriately identified.
- Radioactive materials, narcotics and other hazardous, sensitive and/or dangerous pharmaceutical products, as well as products presenting special risks of abuse, fire or explosion, (e.g. combustible liquids and solids and pressurized gases) should be stored in a dedicated area that is subject to

appropriate additional safety and security measures. Pharmaceutical products should be handled and distributed according to good manufacturing practice.

- Pharmaceutical products should be handled and stored in such a manner as to prevent contamination, mix-ups and cross-contamination.
- A system should be in place to ensure that pharmaceutical products due to expire first are sold and/or distributed first (FEFO). Where no expiry dates exist for the products, the FIFO principle should be applied.
- Broken or damaged items should be withdrawn from usable stock and separated.

Storage areas should provide adequate lighting to enable all operations to be carried out accurately and safely.

1.3.2.3.Storage cconditions :-

Storage conditions for pharmaceutical products should be in compliance with the labeling, which is based on the results of stability testing.

1.3.2.4.Monitoring of storage conditions

Recorded temperature monitoring data should be available for review. The equipment used for monitoring should be checked at suitable predetermined intervals and the results of such checks should be recorded and retained. All monitoring records should be kept for at least the shelf-life of the stored material or product plus one year, or as required by national legislation. Temperature mapping should show uniformity of the temperature across the storage facility. It is recommended that temperature monitors be located in areas that are most likely to show fluctuations.

Equipment used for monitoring should also be calibrated at defined intervals. .**(WHO** Working document QAS/04.068/Rev.2)

1.3.3.2.4. Labeled storage conditions:-

The following table shows the meaning of storage conditions defined in the label of the drug :-

Table (1) storage conditions defined in the label of the drugs in general:-

On the label	Means
Do not store over 30 C°	From +2 C° to + C°30
Do not store over 25 C°	From +2 C° to +25 C°
Do not store over 15 C°	From +2 C° to +15 C°
Do not store over 8 C°	From +2 C° to +8 C°
Do not store below 8 C°	From +8 C° to +25 C°
Protect from moisture	No more than 60% RH in normal storage condition; to be provided to the patient in moisture resistant container.
Protect from light	to be provided to the patient in light resistant container.

(WHO, Quality assurance of pharmaceuticals a compendium guidelines and related materials)

1.3.3.2.5. The actual storage conditions in Khartoum :-

The prevalent storage and handling facilities of the medicinal products within Khartoum province was investigated and inspected with regard to compliance with requirement of good storage practice (GSP) and good distribution practice (GDP)

The following results were obtained:-

1.3.3.2.6Available medicine storage facilities:-

25% are normal stores.

20% are normal stores with domestic refrigerators.

20% are normal stores with specially designed refrigerators for medicines storage.

10%are domestic refrigerators.

5%are cold room with domestic refrigerators.

5% are cold room with normal stores with domestic refrigerators.

5% are normal stores with domestic refrigerators and specially designed refrigerators for medicines storage.

1.3.3.2.7. Location of store:-

30%Industerial area.

10%Residential area.

40%Puplic market area.

20%Hospitals.

1.3.3.2.8 Transportation facilities:-

35%Special container with ice bag and refrigerated vehicles.

55%No facilities.

5% Special container with ice bag and no facilities.

5%Non refrigerated vehicles.

1.3.3.2.9.Electricity availability:-

30%very good.

55%good.

15%poor.

1.3.3.2.10. Temperature control:-

30%very good (controlled and monitored).

40%good (controlled and no monitoring).

30%poor (no monitor and no control)

(Hussein A. H. august 2006)

1.4.Good manufacturing practices (GMP)

It is the part of quality assurance which ensures that products are consistently produced and controlled to the quality standards appropriate to their intended use and as required by the marketing authorization.

.**(WHO** Working document QAS/04.068/Rev.2)

1.5. Climatologically Normals For the period (1971-2000):-

The metrological authority is the main body which is responsible for detecting the temperatures and relative humidity in different areas of Sudan**;** they calculate the mean **t**emperature and relative humidity for each month by taking the average of 30 years. The following tables show the average of temperatures and relative humidity for some selected areas in Sudan:-

Table NO. (2) Expected temperature over a year in Khartoum :-

Month	Mean Temperature C˚		RH%
	Max	Min	
January	30.7	15.6	26
February	32.6	16.8	21
March	36.5	20.3	16
April	40.4	24.1	15
May	41.9	27.3	20
June	41.3	27.6	26
July	38.5	26.2	42
August	37.6	25.6	48
September	38.7	26.3	41
October	39.3	25.9	29
November	35.2	21.0	26
December	31.7	17.0	29
Annual	37.0	22.8	28

Table NO. (3) Expected temperature over a year in Port Sudan:-

Sudan :-

Month	Mean Temperature C˚		RH%
	Max	Min	
January	26.6	19.4	65
February	26.9	18.7	65
March	28.5	19.5	63
April	31.8	21.7	59
May	35.4	24.2	53
June	38.4	26.3	44
July	40.7	28.5	44
August	40.4	29.0	46
September	37.9	27.1	55
October	33.8	25.3	67
November	30.9	23.7	69
December	28.3	21.1	68
Annual	33.3	23.7	53

Table NO. (4) Expected temperature over a year in Dongola:-

Month	Mean Temperature C˚		RH%
	Max	Min	
January	26.5	8.7	35
February	29.3	10.1	29
March	33.7	14.0	23
April	38.9	18.9	19
May	42.2	23.2	17
June	43.1	24.9	17
July	42.6	25.5	20
August	42.2	25.7	21
September	41.8	25.4	21
October	39.0	21.8	23
November	32.1	14.9	30
December	28.2	10.3	35
Annual	36.6	18.6	24

Table NO. (5) Expected temperature over a year in Elgadarif:-

Month	Mean Temperature C˚		RH%
	Max	Min	
January	34.9	17.2	34
February	36.6	18.3	27
March	39.3	21.4	23
April	41.4	24.2	23
May	40.6	25.4	34
June	37.8	23.5	48
July	33.6	21.7	65
August	32.5	21.3	72
September	34.4	21.6	66
October	36.9	22.3	49
November	37.4	21.2	33
December	35.6	18.4	35
Annual	36.7	21.4	42

Table NO. (6) Expected temperature over a year in Elfashir :-

Month	Mean Temperature C˚		RH%
	Max	Min	
January	29.7	13.1	23
February	32.0	15.2	19
March	35.6	18.8	15
April	38.8	12.7	15
May	39.4	24.5	28
June	37.7	24.2	40
July	34.2	22.8	61
August	33.3	22.3	67
September	35.2	22.4	59
October	36.8	22.4	37
November	30.6	18.0	25
December	30.4	14.2	26
Annual	34.7	20.0	35

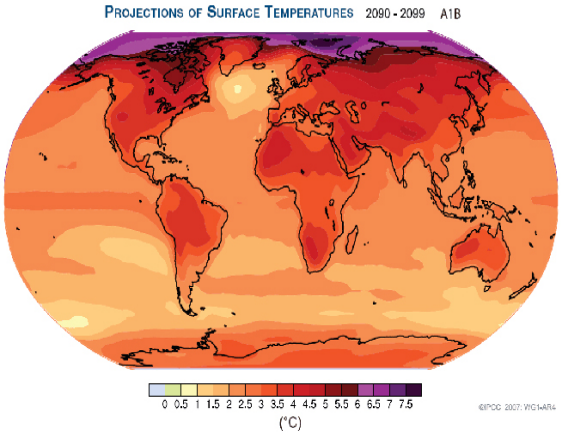
(Metrological authority data sheet 2006)

1.6. Global worming:-

Global Warming is defined as the increase of the average temperature on Earth. As the Earth is getting hotter, disasters like hurricanes, droughts and floods are getting more frequent.

Over the last 100 years, the average temperature of the air near the Earth's surface has risen a little less than 1° Celsius (0.74 ± 0.18°C, or 1.3 ± 0.32° Fahrenheit). The data show that an increase of one degree Celsius makes the Earth warmer now than it has been for at least a thousand years. Out of the 20 warmest years on record, 19 have occurred since 1980. The three hottest years ever observed have all occurred in the last eight years; this phenomenon make us aware to take care about the storage conditions especially that Sudan lie in the area which has great changes in temperatures as show in figure (1).

Figure (1) Map for temperature changes due to global warming:-



Karin Lindinger, Allianz. Com publishing date: April 30, 2007

1.7. Effect of transportation:-

The effect of transportation was already studied using ergometrin, as general feature, of all the pharmaceutical products investigated, no significant degradation was experienced as a result of transportation from Europe to port Sudan. However, a significant decrease in the content occurred immediately after dispatch of samples to Khartoum medical stores, whereupon the content dropped to a value of 89.5%, which is below the USP lower limit.

(I.O.AbuReid etal., 1990.)

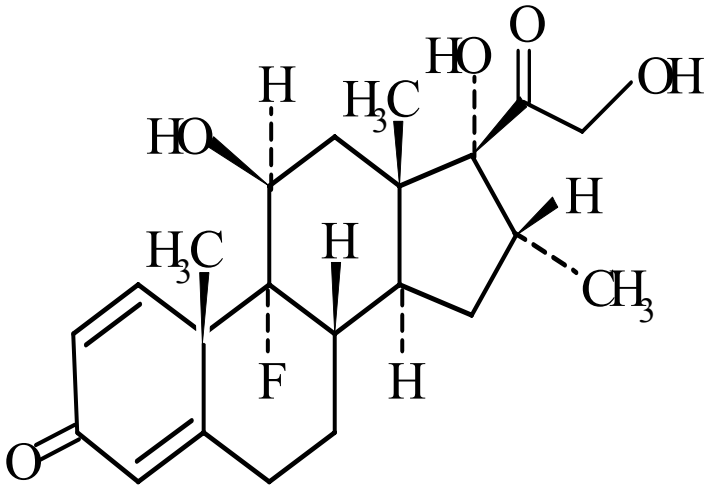
1.8.The targeted drug:-

1.8.1.Dexamethasone:-

Dexamethasone is a synthetic glucocorticoid and an isomer of betamethasone. It is a white crystalline powder with melting point 268 C ° to 271 C°, with decomposition. A solution in dioxan is dextrorotatory.

1.8.1.1. Structure :-

$C_{22}H_{29}FO_5$ ----- 392.41



1.8.1.1. Structure activity relation ship:-

- The OH group at position 17 is very important for anti-inflammatory activity.
- The fluoride in position 9 increase the anti-inflammatory effect.
- The methyl group at position 16 decrease the side effect.

1.8.1.2. Solubility:-

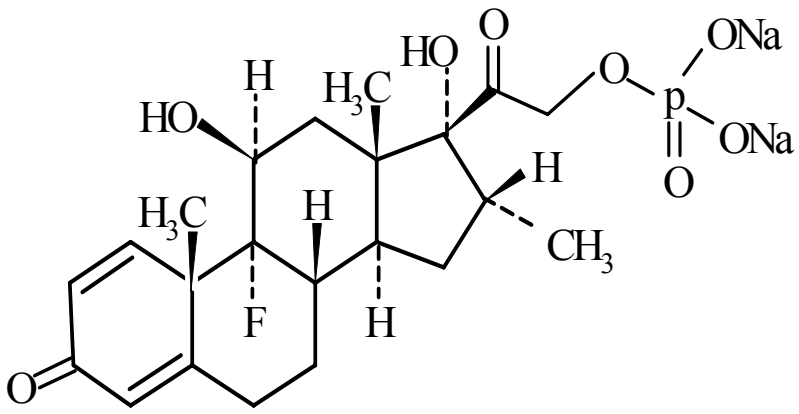
Practically insoluble in water; soluble 1in 42 of ethanol and1in165of chloroform; soluble in acetone; sparingly soluble in methanol; very soluble in ether.

(**Clark´s** analysis of drugs and poisons)

1.8.2. Dexamethasone sodium phosphate :-

1.11.2.1 Structure:-

$C_{22}H_{28}FNa_2O_8P$ ----- 516.41



melting point 233 C ˚ to 235 C˚

1.11.2.2. Solubility :-

Soluble 1in2 of water; sparingly soluble in dehydrated alcohol; practically in soluble in chloroform and ether.

(Walter Lund 12 edition*)*

1.9. Stability of dexamethasone

Dexamethasone sodium phosphate injection should be protected from light and dexamethasone tablets should be stored in well-closed containers protected from light.

(Walter Lund 12 edition)

The stability of dexamethasone has been well studied:-

Sterile dexamethasone acetate suspensions and dexamethasone sodium phosphate injections that had been stored in hospital pharmacies across the United States was studied. Through a voluntary FDA drug stability program, all hospital pharmacies in the United States were asked in October 1981 to complete a response card indicating information about the sterile dexamethasone acetate suspensions and dexamethasone sodium phosphate injections they had in stock. Based on the responses, FDA selected 21 samples of sterile dexamethasone acetate suspensions (representing two manufacturers) and 114 samples of dexamethasone sodium phosphate injection (representing 11 manufacturers). These samples were analyzed for identification, pH, and strength. All samples of sterile dexamethasone acetate suspension met USP requirements. Eleven samples of dexamethasone sodium phosphate injection representing 10 lots from three manufacturers failed USP assay requirements for strength. All samples that failed to meet strength requirements showed evidence of degradation by oxidation. Sterile dexamethasone acetate suspensions appear to be stable when stored under actual marketplace conditions, but there is a problem with the shelf-life stability of dexamethasone sodium phosphate injections made by some manufacturers. (Coffman HD,etal. , 1983 Volume 40)

A commercial sample of dexamethasone sodium phosphate solution for injection was found to contain 56% of the labeled concentration and to be extensively contaminated with a white insoluble solid, which was identified as a mixture of the 16α- and 16β-methyl epimers of 9-fluoro-11β-hydroxy-16methylandrosta-1,4-Diene-3,17Dione after examination by high performance liquid chromatography ,TLC,UV, and IR spectrophotometry.

(Eric C.Juenge and James F.Brower 1979)

Dexamethasone tablets was assayed by direct ultraviolet absorption at 240 nm as well as colorimetrically following the official method .The tablets were similarly treated ; the direct spectrophotometric determination for a methanolic extract gave higher result than the colorimetric method, this is due to the decomposition product which absorbs at that wave length , This decomposition product was separated using TLC methods.

(S.K. Wahba, etal., July1968)

Chapter(2)
Aim of
The Research

1- Depending on the reported stability studies of dexamethasone and dexamethasone sodium phosphate it was thought of great interest and importance to check the stability of formulations of dexamethasone and dexamethasone sodium phosphate available in Sudan.

2- The study is aimed to cover the effect of actual storage conditions on the formulations available in pharmacies and drug stores in different areas in Sudan.

3- The outcome of point (1)and (2) are :

(A) To find out the suitable storage conditions for such life saving drugs.

(B) Make authorities appreciate importance of such stability studies.

Chapter 3
Experimental

3.1.Instrumentation:-

3.1.1 Four decimal sensitive balance (ADAMS) was used for weighing all materials.

3.1.2 Double beam UV-VIS spectrophotometer 1700 Shimadzu Pharmaspec , was used for quantitative analysis of dissolution of dexamethasone tablets. The spectral width was (2 nm) and the wavelength scanning speed was medium . The absorption spectra of test and reference solutions were recorded in 1 Cm quartz cells at the wave length 240 nm .

3.1.3. Two High performance liquid chromatography were used:-

3.1.3.1. Knauer which was connected to UV/VIS detector (K-2600),HPLC pump K-1001 ,and brackets injector valve with 25µL sample loop and laboratory computing integrator .

3.1.3.2. Shimadzu which was connected to UV/VIS detector (SPD-10AVP) , with Shimadzu auto sampler injector(SIL -10ADVP) ,Shimadzu (SCL-10 AVP)system controller ,Shimadzu liquid chromatograph (LC-10 AT VP)and Shimadzu degasser(DG-14A) .

3.1.4. The separations were performed on Thermo column packed with octadeceyl -silane chemically bonded to silica 250*4.6 mm at room temperatures .

3.1.5. ERWEKA DT6 dissolution tester (apparatus1).

3.1.5. Steam bath.

3.1.6. FN400 Oven.

3.1.7. Power sonic 405 (Micro process controlled Bench-top ultrasonic cleaner).

3.1.8. Jenway 3510 pH meter.

3.2.Materials

3.2.1. Formulations selected to be analyzed:-

3.2.1.1 Dexamethasone sodium phosphate. Injection 4mg/ml :-

2.1.1. Manufacturer:-Shanghai pharmaceutical co., LTD.

2.1.2. Batch number :- 070408.

2.1.3. Manufacture date :-**04/2007**.

2.1.4. Expiry date:-**04/2010**.

2.2. **Dexamethasone tablet 1.5mg/tablet** :-

2.2.1. Manufacturer:-Organon and Medico labs

2.2.2. Batch number:- 152365 (Organon) and 246 (medico).

2.2.3. Manufacture date :- _(Organon) and11/2007 (medico).

2.2.4. Expiry date:- 09/2009(Organon) and11/2010 (medico).

2.3. Dexamethasone sodium phosphate eye ointment 0.05% :

2.3.1. Manufacturer :-Alcon cusi

2.3.2. Batch number :- 5CTB1A.

2.3.3. Manufacture date :- 12/2005.

2.3.4. Expiry date:- 12/2010**.**

2.4. Dexamethasone oral solution10mg/100ml:-

2.4.1. Manufacturer:- The Arab Drug company.

2.4.2. Batch number:- 730222.

2.4.3. Manufacture date:- 6/2007.

2.4.4. Expiry date:- 6/2010.

3.3. Study design and sampling :-

Each dosage form was represented by certain drug which is stable in normal controlled room temperature and humidity but when it is exposed to drastic conditions it may show changes due to instability . To exclude the effect of the drug as one factor the same drug in different dosage forms were selected. Dexamethasone was chosen in this work for tablets, ointments ,syrups and dexamethasone sodium phosphate for injections. Samples were collected directly from manufacturer and the central medicine stores (C M S) ; samples were analyzed first as zero time , then at different time intervals according to the following protocol :-

1-One batch of each drug formulation was stored in stability cabinet at temperature of 30 ºC and 70% R H (Registration requirement).

2- Part of the same batch stored in the cabinet was distributed to different areas in Sudan ; these areas (Dongola, Port Sudan, Elfashir ,Elgadarif and Khartoum) were selected to cover the different climatic conditions in Sudan . The distribution took place by controlled temperature vehicles and stored in these areas at actual storage conditions in retail pharmacies, and they were collected at the time of test and send by plane or controlled temperature vehicles as follows:-

- after 4 month
- after 8 month
- after 12 month and so on till the samples show significant changes during the shelf life.

The same batches were stored in well controlled conditions as controls.

3.4.Analysis of samples:

Samples were analyzed according to the USP HPLC methods to assess the affect of the real storage conditions on stability of the different dosage forms.

All methods used were USP 2007 except for the dissolution test for the dexamethasone tablets that because one of the reagents needed for the test was not available (tetrazolium blue).

3.4.1.Reagents :-

3.4. 1.1. Samples and standard preparations :-

All reagents used for sample and standard preparations were of analytical grade obtained from different sources as follows:-

Table (7) reagents used for samples and standards preparation

No	Reagent	Source
1	Methanol	Romil pure chemistry
2	Acetonitrile	Romil pure chemistry
3	Anhydrous acetic acid	Scharlue
4	Chloroform	BDH laboratories supplier
5	Hydrochloric acid 37%	BDH laboratories supplier
6	Alcohol	Romil pure chemistry
7	Monobasic Potassium phosphate	Loba chemie
8	Monobasic sodium phosphate	Loba chemie
9	glacial acetic acid	Scharlue

3.4.1.2 For dexamethasone sodium phosphate injection:-

For sample and standard preparation suitable degassed solution of 0.01M monobasic potassium phosphate in a mixture of methanol: water (1:1) was used to dilute the injection to the specified concentration.

3.4.1.3. For dexamethasone tablets :-

For sample and standard preparation dilute methanol (1 in 2) in water was used.

3.4.1.4. For dexamethasone sodium phosphate eye ointment:-

For sample and standard preparation chloroform and alcohol-aqueous phosphate buffer were used.

3.4.1.5. For dexamethasone oral solution:-

For sample and standard preparations a mixture of methanol and water (1:1) was used.

3.4.1.2. Mobile phases preparations :-

Acetonitrile and methanol used for mobile phases preparations were of HPLC grade:-

3.4.1.2. 1. For dexamethasone sodium phosphate injection:-

A filtered and degassed solution of 0.01M monobasic potassium phosphate in a mixture of methanol: water (1:1) was prepared.

3.4.2. 2 for dexamethasone tablets:-

Filtered and degassed aqueous solution of acetonitrile **(**1 in 3) in water was used**.**

3.4.2.3. for dexamethasone sodium phosphate eye ointment:-

Filtered and degassed solution of methanol and 0.05M phosphate buffer (6.9 gram of monobasic sodium phosphate was dissolved in a liter of water) in a ratio of 52:48 was used.

3.4.2.4 For dexamethasone oral solution:-

A filtered and degassed mixture of methanol: water: glacial acetic acid (55:43:2) was used.

3.4.1.3. Standard solutions preparations :-

3.4.3.1. For dexamethasone sodium phosphate injection assay:-

An accurately measured weight of dexamethasone sodium phosphate standard (0.01gram) was dissolved in 100 ML diluting solution.

3.4.3.2.For dexamethasone tablet assay:-

An accurate weight of dexamethasone standard (0.01 gram) was transferred in to 100 ML volumetric flask, dissolved and dilute to volume with dilute methanol (1 methanol in 2 water).

3.4.3.3 For dexamethasone sodium phosphate eye ointment assay:-

An accurate weight of dexamethasone sodium phosphate standard (0.01gram) was transferred in to 100 ML volumetric flask, dissolved and dilute to volume with alcohol aqueous phosphate buffer, 5ML of this solution was transferred to 50ML volumetric flask and dilute to volume with alcohol with aqueous phosphate buffer.

3.4.3.4 For dexamethasone oral solution assay:-

An accurate weight of dexamethasone standard (0.01gram) was transferred in to 100 ML volumetric flask, dissolved and diluted with mixture of methanol: water (1:1), 5ML of this solution was transferred to10ML volumetric flask and diluted to volume with the same solvent.

3.4.1.4.Sample preparation:-

3.4..4.1. Dexamethasone sodium phosphate injection assay:-

An accurately measured volume of injection (2ML) was diluted to 100 ML with diluting solution.

3.4.4.2 Dexamethasone tablets:-

3.4..4.2.1 Dissolution test:

One tablet was introduced to each dissolution tester vessels which was filled with medium of 500 ml hydrochloric acid (37 %) 1 in 100 and basket (apparatus 1) was operated at 100 r.p.m. for 45 minutes at 37C°.

(50 ml) of Filtered aliquot of dissolution media was extracted with three (15 ml) of chloroform, the combined extracts were evaporated on steam bath just to dryness, cooled and dissolved in (10 ml) of methanol.

3.4.4.2.2. Dexamethasone tablets assay:-

10 tablets were accurately weighted, transferred to a mortar and finely powdered. A mass containing 5 mg of dexamethasone was accurately weighted and transferred in to50 ml volumetric flask, suspended in 30 ml of dilute methanol (1in2), sonicated for about 2 minutes and shacked by mechanical means for 30 minutes , then diluted with the same solvent to volume . The solution was filtered through Whatman No 40 filter paper.

3.4.4.3. **Dexamethasone sodium phosphate ointment assay:-**

An accurate weight of ointment (2.5 gram) was transferred to 100 ml beaker; 10ml of alcohol aqueous phosphate buffer was added and heated just to boiling and cooled .The contents of the beaker were transferred to separatory funnel containing 45ml of chloroform ;after shaking for one minute the upper layer was collected in 25ml volumetric flask and the remaining chloroform was extracted with two 5 ml of the buffer and the upper layer was collected in the same 25mlvolumetric flask , the volume was completed with same solvent and mixed . The solution was filtered through Whatman No 40 filter paper.

3.4.4.4. Dexamethasone oral solution:-

An accurately measured volume of oral solution was transferred into 50 ml volumetric flask and diluted to volume with mixture of methanol water (1:1).

3.5. Procedures:-

3.5.1. For dexamethasone sodium phosphate injection :-

An equal volumes (25µl) of samples and standard preparations were injected into the HPLC system at room temperature connected with Thermo column 4.6mm *250mm with particle size 5µ packed with ODS(octadecyl silane chemically bonded to porous silica) and equipped with UV detector capable of monitoring absorptions at 254 nm with flow rate 1.5 ml/minute ; the peak retention time was about 5 minutes**.**

3.5.2 Dexamethasone tablets:-

3.5.2. 1.Dissolution test:-

The UV absorbance was recorded at 240 nm and 385 was used as value of ($A^{1\%}_{1}$ Cm) using methanol as a blank.

3.5.2.2. **Dexamethasone tablets assay**:-

An equal volumes (25µl)of samples and standard preparations were injected in to HPLC at room temperature connected with Thermo column 4.6mm *250mm with particle size 5µ packed with ODS(octadecyl silane chemically bonded to porous silica) and equipped with UV detector capable of monitoring absorptions at (254 nm) with flow rate 2ML/minute, the peak retention time was 5 minutes.

3.5.3. **Dexamethasone sodium phosphate ointment assay:-**

An equal volumes (25µl) of samples and standard preparations were injected in to HPLC at room temperature connected with Thermo column 3.9 mm *300mm backed with ODS(octadecyl silane chemically bonded to porous silica and equipped with UV detector capable of monitoring absorptions at 254 nm with flow rate 1.5ML/minute, the peak retention time was 5 minutes**.**

3.5.4. Dexamethasone oral solution assay:-

An equal volumes (25µl) of samples and standard preparations were injected in to HPLC at room temperature connected with Thermo column 4.6mm *250 Mm with particle size 5µ packed with ODS(octadecyl silane chemically bonded to porous silica) and equipped with UV detector capable of monitoring absorptions at 254 nm with flow rate 2ml/minute, the peak retention time was 5 minutes**.**

3.6.Validation of the non official methods(dissolution for tablets) :-

As mentioned before the dissolution test of dexamethasone tablets was modified due to unavailability of tetrazolium blue, so we have to validate this modification.

3.6.1. For dexamethasone tablet dissolution :-

3.6.1.1. Linearity:-

The same procedure as in the dissolution was followed to prepare different solutions with concentrations of 15.0 , 10.0 , 6.0 , 3.0 , 1.5 µg /ML . The UV absorbance was recorded at (240 nm) and 385 was used as($A^{1\%}_{1}$ Cm) and methanol as blank**.**

3.6.1.2. Selectivity:-

Solutions of dexamethasone and prednisolone and clobetasone standards (which have structures very close to each other) were prepared in methanolic hydrochloric acid (1 in 100) and the ultraviolet spectrums

were recorded between 200 and 400 and methanolic hydrochloric acid was used as blank .

3.6.1.3. Limit of quantitation :-

Concentrations of 15.0, 6.0, 3.0, 1.5 µg /ML of dexamethasone tablets were prepared from dissolution media after extraction and the ultraviolet absorbance was recorded at 240 nm and 385 was used as($A^{1\%}_{1}$ Cm) using methanol as blank**.**

3.6.1.4. Sensitivity:-

3.6.1.4.1. The ultraviolet absorbance:-

The UV absorbance of the dissolution media and dexamethasone standard prepared in methanolic hydrochloric acid was directly recorded at 240 nm.

The UV absorbance of the extracted dissolution media was recorded at (240 nm) and 385 was used as ($A^{1\%}_{1}$ Cm) and methanol as blank then compared with standard solution in methanol prepared in equivalent concentrations.

The UV absorbance of the dissolution media containing dexamethasone and dexamethasone standard prepared in methanolic hydrochloric acid was directly recorded at (254 nm).

3.6.1.4.2. The HPLC method:-

The dexamethasone standard and dissolution media of sample was prepared in concentrations of 3.0 µg /ml, 25 µl was directly injected in to HPLC system at room temperature connected with Thermo column 4.6mm *250mmwith particle size 5µ packed with ODS (octadecyl silane chemically bonded to porous silica) and equipped with UV detector capable of monitoring absorptions at (254 nm) with flow

rate 2ml/minute, the beak appeared after 5 minutes when filtered and degassed aqueous solution of acetonitrile **(**1 in 3) was used as mobile phase.

The extracted dissolution media and dexamethasone standard were prepared in concentrations of 3.0 µg/ml, 25 µl was directly injected in to HPLC at room temperature connected with Thermo column 4.6Mm *250Mm packed with ODS(octadecyl silane chemically bonded to porous silica) and equipped with UV detector capable of monitoring absorptions at (254 nm) with flow rate 2ml/minute, the beak appeared after 5 minutes when filtered and degassed aqueous solution of acetonitrile **(**1 in 3) was used as mobile phase.

3.6.1.5 Reproducibility:-

The dissolution test was repeated three times in different days.

3.6.1.6. Accuracy:-

The dissolution test for samples was done and concentrations of 3.0 µg/ml (90%), 3.3µg /ml (100%) and 3.6. µg /ML (110%) were prepared then the UV absorbence compared with the theoretical one.

Chapter (4) Results and calculations

The following tables and figures are obtained from analysis of different dosage forms of dexamethasone and dexamethasone phosphate marketed in Sudan using the analysis methods mentioned in chapter (3)

4.1.Results for dexamethasone sodium phosphate injection assay:

The following equation was used to calculate the % content of dexamethasone sodium phosphate injections assay :-

% content dexamethasone sodium phosphate injection assay =

AUC of sample * Concentration factor * potency of standard /AUC of Standard

Table 8:
Follow up HPLC results of dexamethasone injections assay over year from Dongola, Elgadarif and Port Sudan:

	Dongola	RSD	Elgadarif	RSD	Port-Sudan	RSD
Zero time	106.3% N=3	0.0127	106.3% N=3	0.0127	106.3% N=3	0.0127
4 month	98.8% N=3	0.02122	105.2% N=3	0.0254	105.3% N=3	0.02262
8 month	85.2% N=3	0.02804	80.7% N=3	0.02303	81.3% N=3	0.00406
12month	81.2% N=3	0.0262	79.0% N=3	0.0212	83.0% N=3	0.02625

Figure 4 Follow up HPLC results of dexamethasone injections assay over year from Dongola, Elgadarif and Port Sudan:

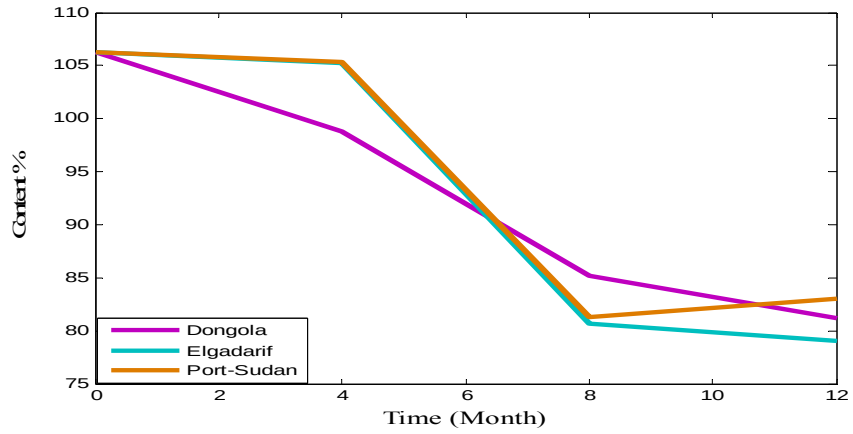


Table 9: Follow up HPLC results of dexamethasone injections assay from Elfashir, Khartoum and Stability cabinet:

	Elfashir	RSD	Khartoum	RSD	Stability cabinet	RSD	C.D.S.
Zero time	106.3% N=3	0.0127	106.3% N=3	0.0127	106.3% N=3	0.0127	106.3% N=3
4 month	103.6 % N=3	0.00990	99.4% N=3	0.00525	96.9% N=3	0 .0063	-
8 month	84.8% N=3	0.00133	84.03% N=3	0.01773	90.3 0% N=3	0.00096	-
12month	84.6% N=3	0.02265	79.3% N=3	003336	86.8% N=3	0.03888	103.1%

Figure 5 Follow up HPLC results of dexamethasone injections assay from Elfashir, Khartoum and Stability cabinet

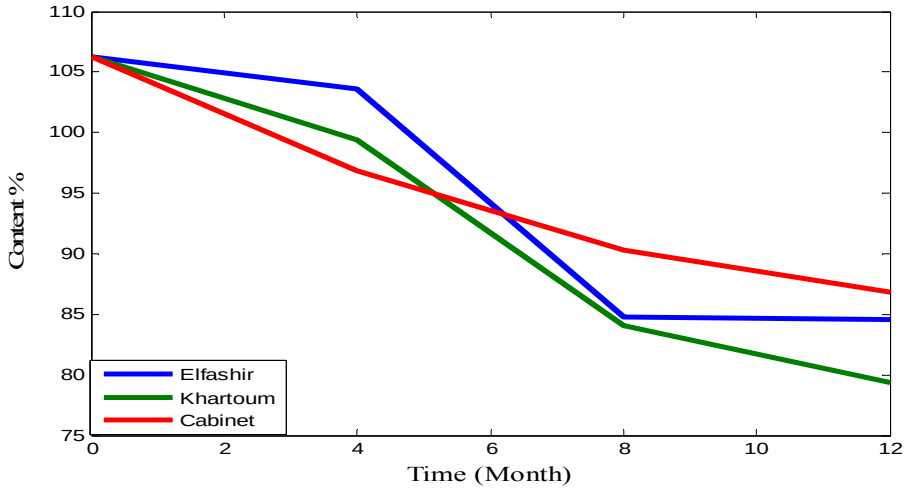
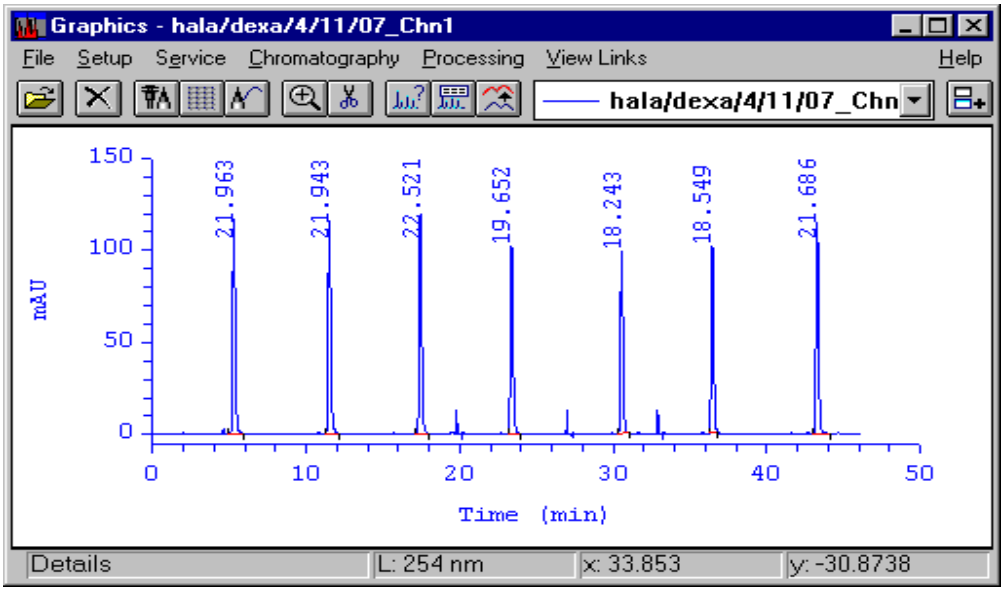


Figure 6: Examples of HPLC chromatogram for dexamethasone sodium phosphate injection:

- **HPLC chromatogram for initial assay:**



- **HPLC chromatogram for assay after 8 month from Elgadarif and Dongola(concentration factor=1.61):**

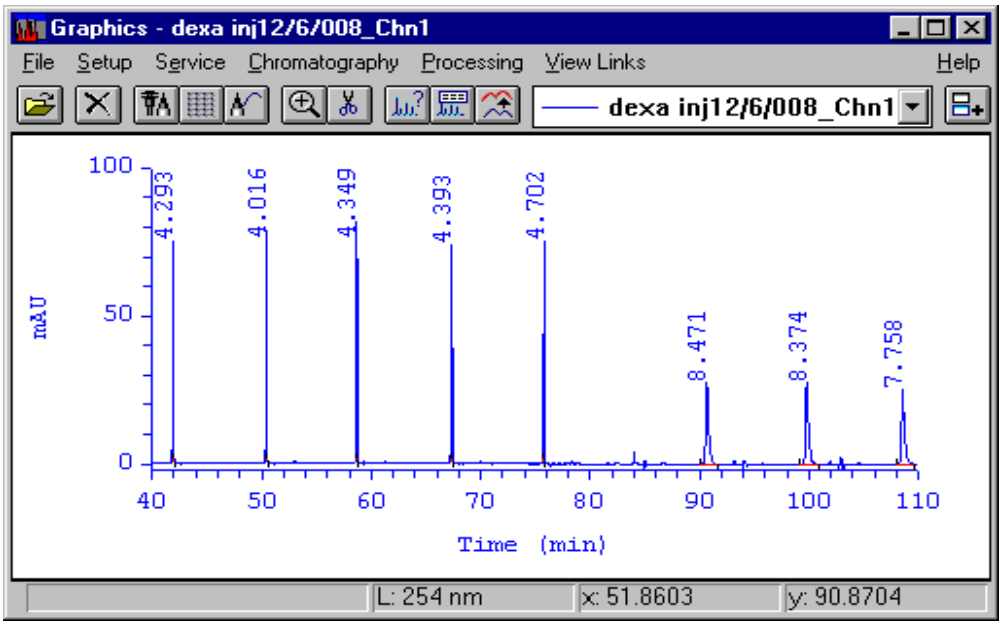


Figure 7 :
Comparison between assay results of dexamethasone sodium phosphate injections from different areas over 12month

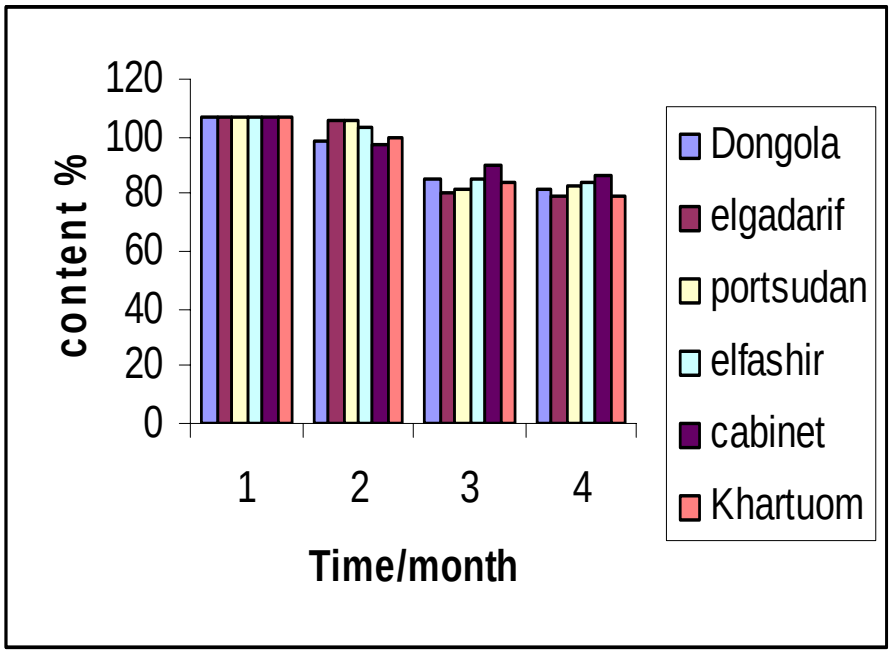
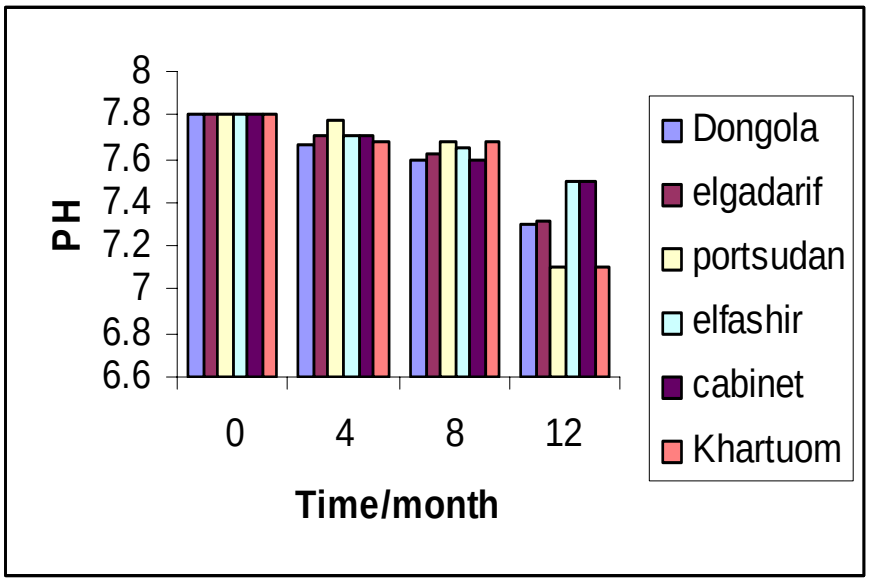


Table 10: follow up results of dexamethasone sodium phosphate injections PH over a year :

	Dongola	Elgadarif	Port-Sudan	Elfashir	Khartoum	Stability cabinet
Zero time	7.80	7.80	7.80	7.80	7.80	7.80
4 months	7.67	7.70	7.78	7.70	7.68	7.70
8months	7.60	7.62	7.68	7.65	7.68	7.60
12months	7.30	7.31	7.10	7.50	7.10	7.50

Figure 8

Comparison between PH results of dexamethasone sodium phosphate injections from different areas over 12month:



4.2.Results for dexamethasone tablets :

The following equation was used to calculate the % content of dexamethasone tablets assay :-

% content dexamethasone tablet =

AUC of sample*Concentration factor*potency of standard /AUC of Standard

Table 11 : Follow up Results of dexamethasone tablets

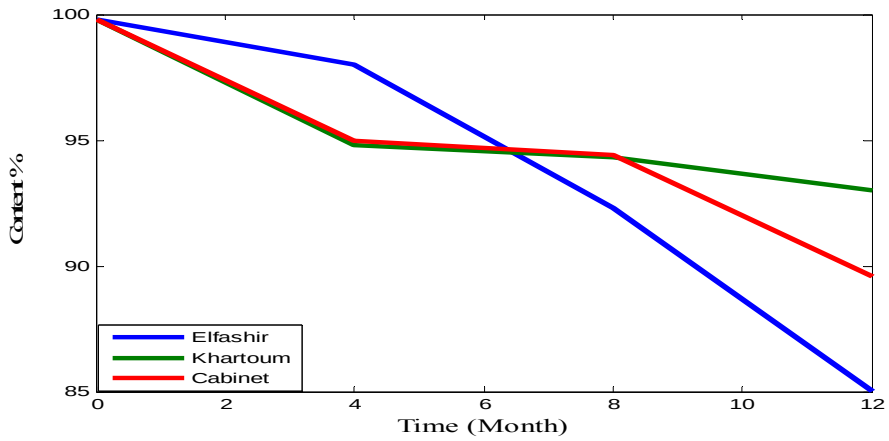
	Elfashir	RSD	Khartoum	RSD	Stability cabinet	RSD
Zero time	99.8% N=3	0.01629	99.8% N=3	0.01629	99.8% N=3	0.01629
4 month	98.0% N=3	0.017977	94.8% N=3	0.0280	95.0% N=3	0.006702
8 month	92.3% N=3	0.00533	94.3% N=3	0.001731	94.4% N=3	0.001737
12 month	85.04%	.001325	93.0% N=3	0.001967	89.6% N=3	0.000407

(oradexone)assay from Elfashir, Khartoum and Stability cabinet:

	N=3					

Figure 9 Follow up Results of dexamethasone tablets (oradexone) assay from Elfashir, Khartoum and Stability cabinet:

	Dongola	RSD	Elgadarif	RSD	Port-Sudan	RSD
Zero time	99.8% N=3	0.01629	99.8% N=3	0.01629	99.8% N=3	0.01629
4 moths	97.0% N=3	0.01162	96.8% N=3	0.005056	95.0% N=3	0.01544
8months	93.5%	0.006018	90.9% N=3	0.019765	93.0% N=3	0.02114



	N=3					
12months	90.8% N=3	0.008166	90.2% N=3	0.002563	86.8% N=3	0.003156

Table 12: Follow up results of dexamethasone tablet (oradexone) assay from Dongola, Elgadarif and Port Sudan

Figure 10. Follow up results of dexamethasone tablet (oradexone) assay from Dongola, Elgadarif and Port Sudan .

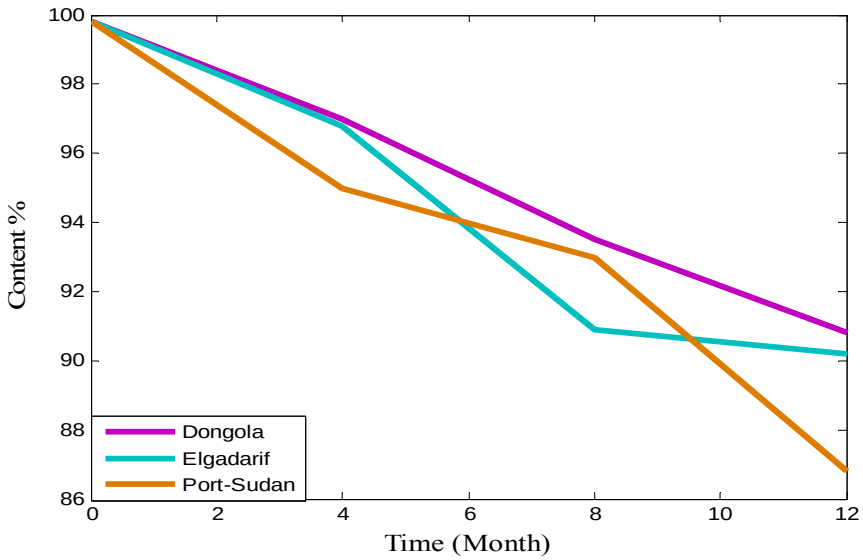


Table13 : Results of dexamethasone tablets assay from another company(Dexamed):

	Company store	Pharmacy
Zero time	104.2	104.2
6 month	90.4	90. 3

Table14:Follow up results of dexamethasone tablets (oradexone) dissolution from Dongola

Zero time	101.2	98.3	98.5	97.4	104.7	97.4
4 months	120.0	116.2	125.3	130.0	140.7	145.1
8months	151.5	173.1	128.2	157.4	151.5	216.4

Table15: Follow up results of dexamethasone tablets (oradexone) dissolution from Khartoum:-

Zero time	98.3	98.5	97.4	104.7	97.4	101.2
4month	173.1	194.8	123.3	116.8	121.3	145.0
8month	189.7	190.8	154.3	133.4	168.5	165.8

Table 16:.Follow up results of dexamethasone tablets (oradexone) dissolution From Elfashir:

ZEROTIME	98.3	98.5	97.4	104.7	97.4	101.2
4 MONTH	163.9	171.4	172.6	152.5	166.2	133.5
8MONTH	177.9	185.7	200.8	158.4	172.5	180.4

Table17:Follow up results of dexamethasone tablets (oradexone) dissolution From Elgadarif :-

Table18:Follow up results of dexamethasone tablets (oradexone) dissolution From stability cabinet :-

ZERO TIME	101.2	98.3	98.5	97.4	104.7	97.4
4 MONTH	160.2	185.4	140.3	100.1	115.0	125.6
8MONTH	186.1	454.5	199.1	80.0	97.4	93.5

ZEROTIME	98.3	98.5	97.4	104.7	97.4	101.2
4 MONTH	138.5	116.8	168.8	123.8	121.2	129.8
8MONTH	140.9	135.7	167.6	120.8	144.0	180.6

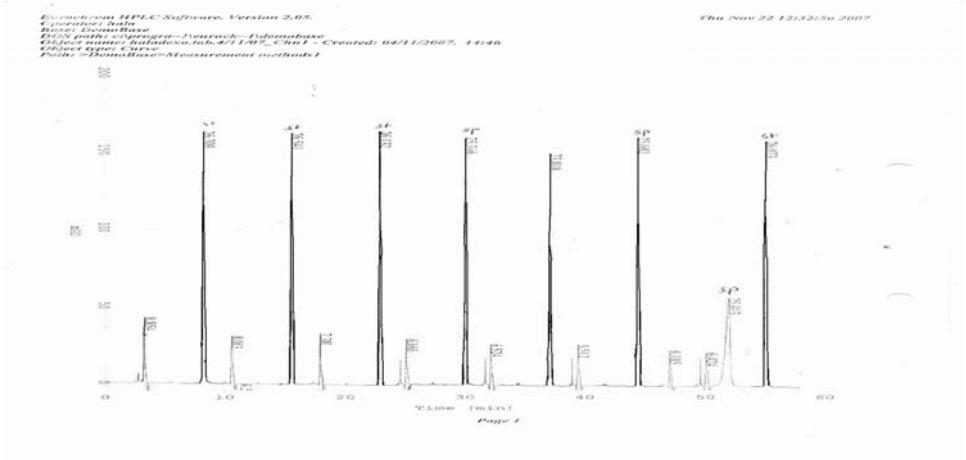
Table 19:Follow up results of dexamethasone table

(oradexone) dissolution from Port Sudan:-

ZERO TIME	98.5	97.4	104.7	97.8	101.2
4 MONTH	125.8	111.3	121.5	150.6	130.0
8 MONTH	161.5	171.4	171.7	172.7	137.8

Figure 11.: Examples of HPLC chromatogram for dexamethasone tablet:

- **HPLC chromatogram for initial assay:**



- **HPLC chromatogram for assay after 8 month from Khartoum**
-

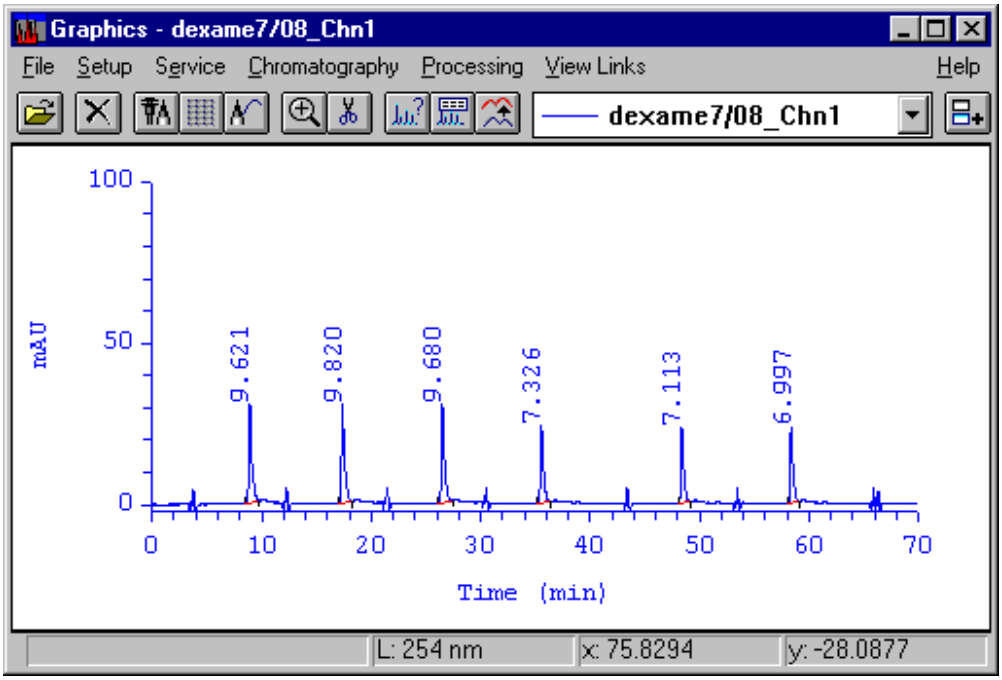
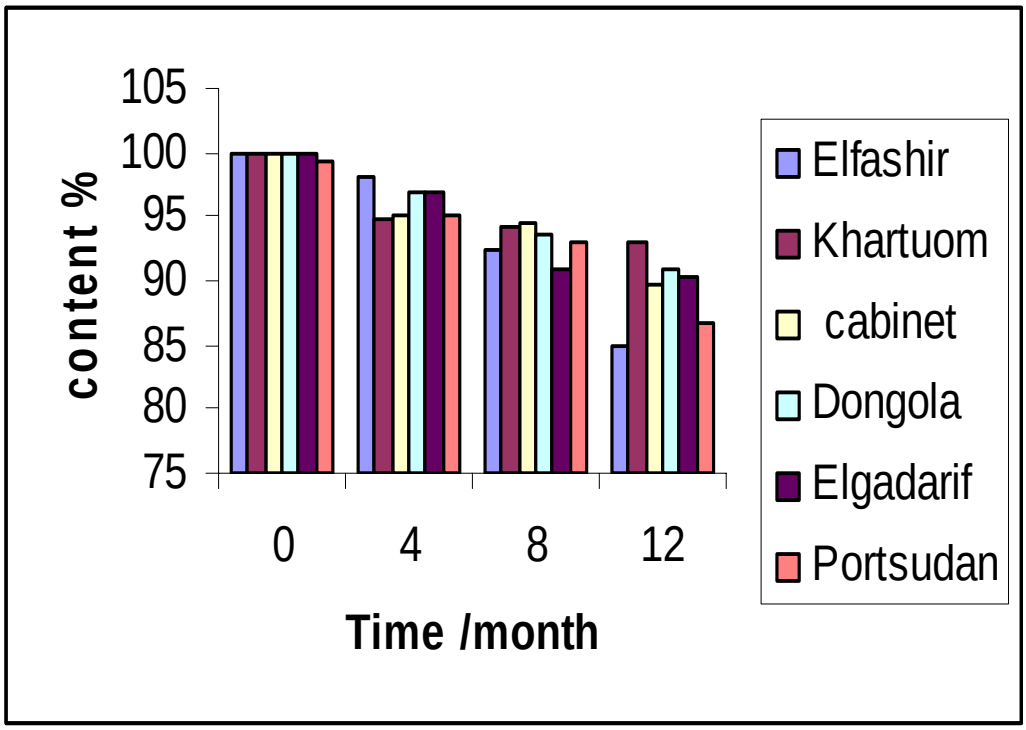


Figure 12. :Comparison between dexamethasone tablets assay from different areas over 12month



4.3. For dexamethasone ointment:

The following equation was used to calculate the % content of dexamethasone ointments assay :-

% content dexamethasone ointment =

AUC of sample*Concentration factor/AUC of Standard

Table 20 Follow up results for dexamethasone ointments assay from Elfashir, Khartoum and Stability cabinet :

	Elfashir	RSD	Khartoum	RSD	Stability cabinet	RSD
Zero time	110.6% N=3	0.015178	110.6% N=3	0.015178	110.6% N=3	0.015178
4 month	105.2 % N=3	0.01622	107.6% N=3	0.017778	110.0% N=3	0.02291
8 month	101.5% N=3	0.004849	107.5% N=3	0.009834	107.4% N=3	0.0184849
12month	101.2% N=3	0.00629	105.1% N=3	0.0023	104.5% N=3	0.00871

Figure13. Follow up results for dexamethasone ointments assay from Elfashir, Khartoum and Stability cabinet :

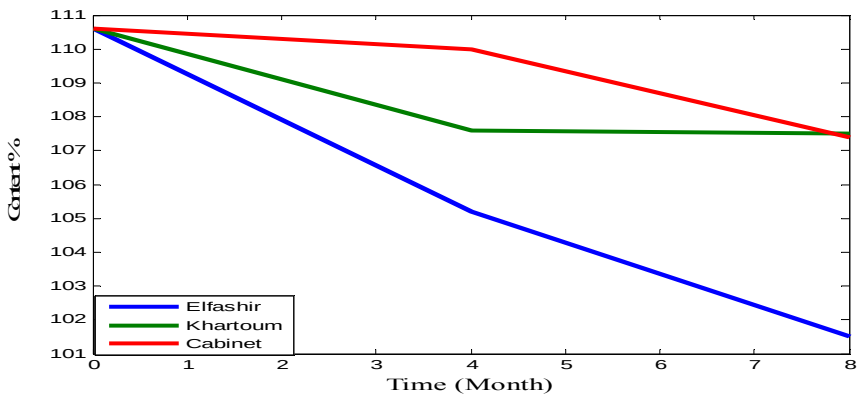


Table 21:

Follow up results of dexamethasone ointments assay from Port Sudan, Elgadarif and Dongola

	Port Sudan	RSD	Elgadarif	RSD	Dongola	RSD
Zero time	110.6% N=3	0.01517	110.6% N=3	0.01517	110.6% N=3	0.01517
4 month	110.0% N=3	0.021001	109.1% N=3	0.01712	109.3% N=3	0.01590
8 month	107.9% N=3	0.01740	107.6% N=3	0.00212	108.9% N=3	0.01215
12month	107.6 N=3	0.01274	98.16 N=3	0.02701	108.1 N=3	0.00983

Figure 14 Follow up results of dexamethasone ointments assay from Port Sudan, Elgadarif and Dongola

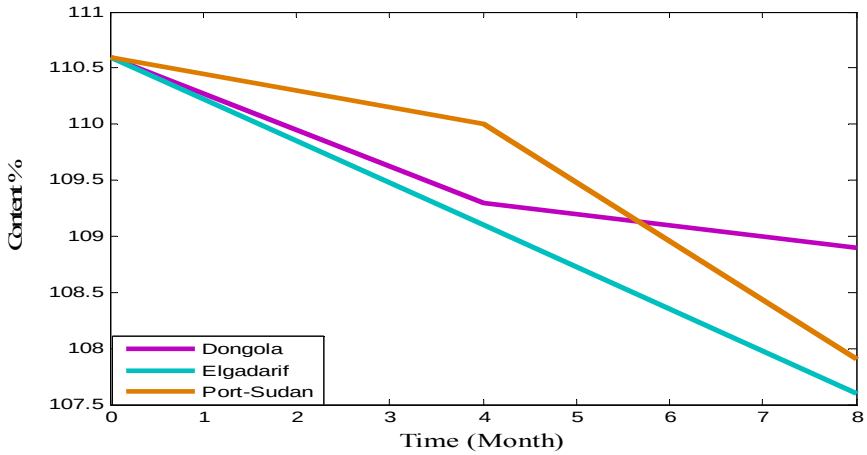
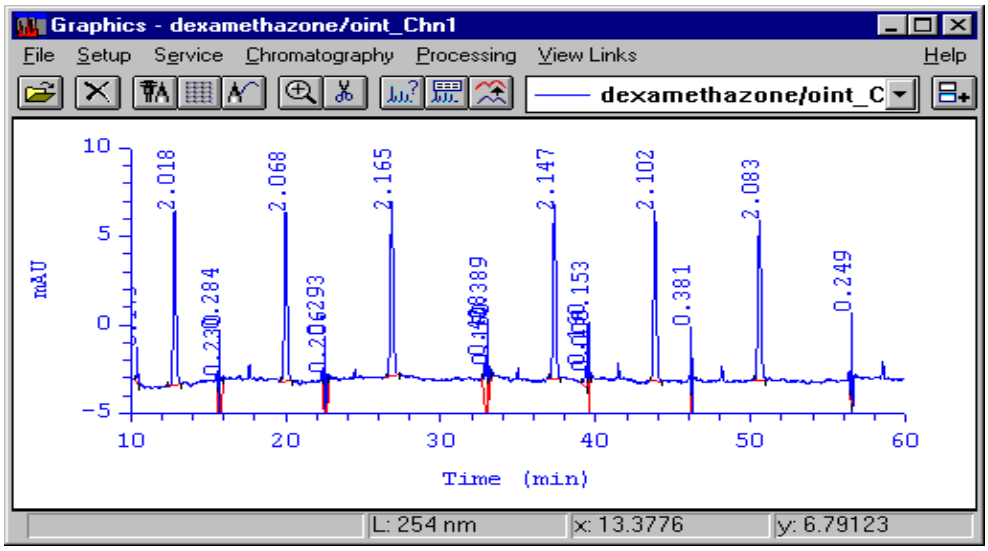


Figure 4.12 : Examples of HPLC chromatogram for dexamethasone ointment

- **HPLC chromatogram for initial assay**



- **HPLC chromatogram for assay after 8 month from cabinet and Alfashir :**

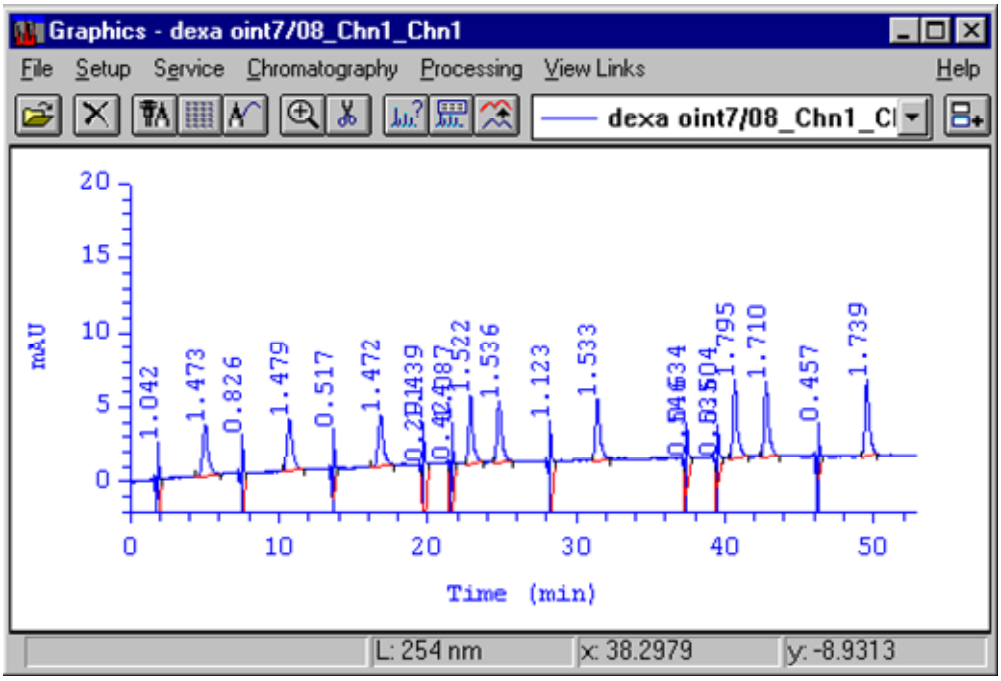
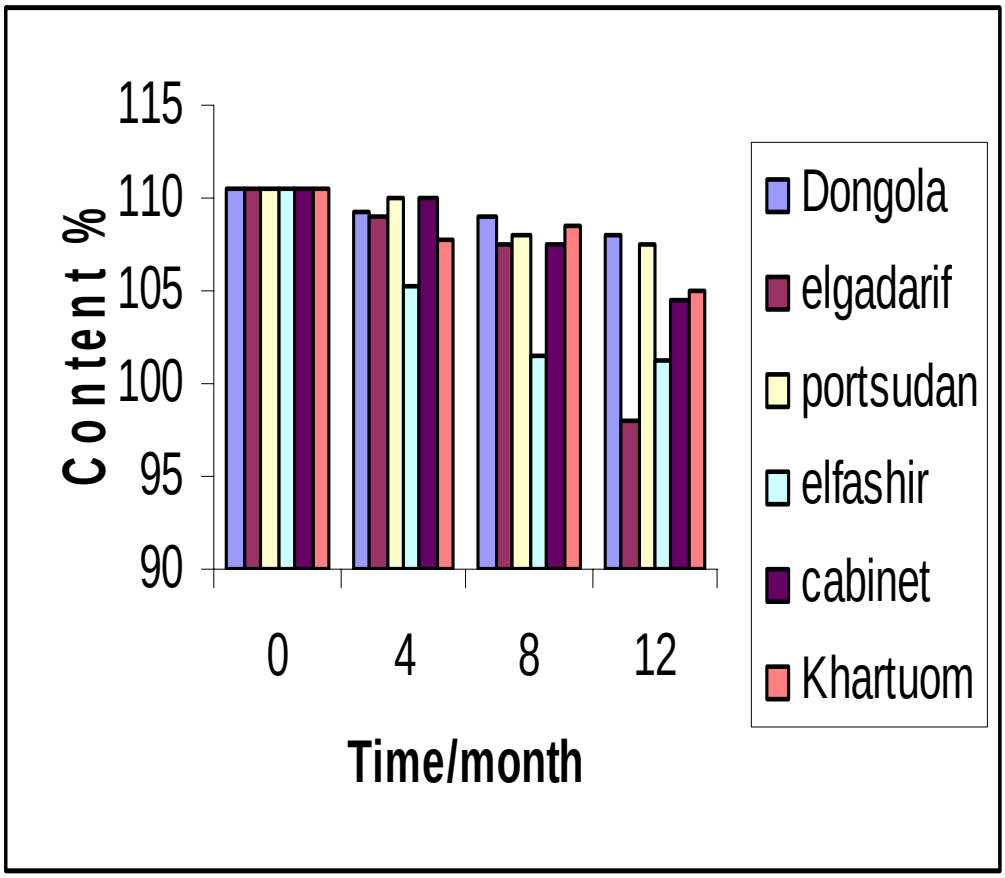


Figure 16. :
Comparison between assay results of dexamethasone ointments from different areas over 12month



4.4.For dexamethasone oral solutions :

The following equation was used to calculate the % content of dexamethasone oral solutions assay:-

% content dexamethasone oral solutions =

AUC of sample*Concentration factor*potency of standard /AUC of Standard

Table (22):

Follow up results of dexamethasone oral solutions assay from Dongola, Elgadarif, Port Sudan and company store:

	Dongola	RSD	Elgadarif	RSD	Port-Sudan	RSD	company store
Zero time	100.2% N=3	0.008411	100.2% N=3	0.008411	100.2% N=3	0.008411	100.2%
4 months	89.8% N=3	0.016301	91.8% N=3	0.003677	92.5% N=3	0.002658	-
8months	76.4% N=3	0.028233	78.4% N=3	0.007199	77.9% N=3	0.012921	-
12months	64.4% N=3	0.026822	64.7% N=3	0.00191	63.5% N=3	0.01348	94.5%

Figure17 Follow up results of dexamethasone oral solutions assay from Dongola, Elgadarif, Port Sudan and company store:

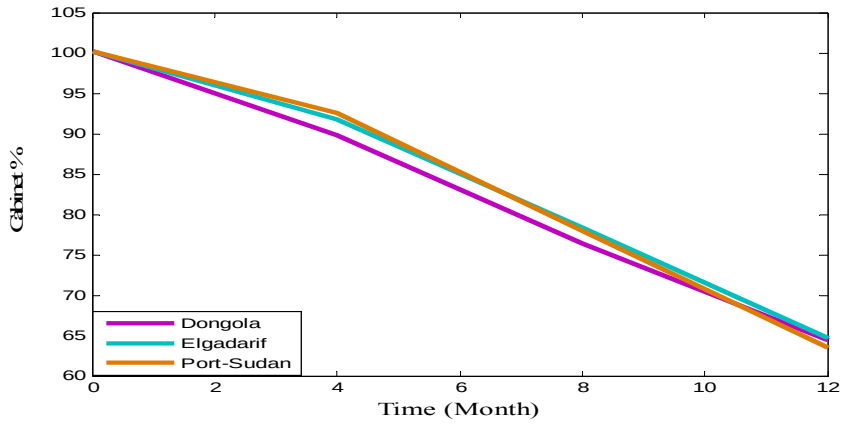


Table 23 Follow up results of dexamethasone oral solutions assay from Elfashir, Khartoum and Stability cabinet

	Elfashir	RSD	Khartoum	RSD	Stability cabinet	RSD
Zero time	100.2% N=3	0.008411	100.2% N=3	0.008411	100% N=3	0.008411
4 month	87.9% N=3	0.002085	94.2% N=3	0.008764	94.9% N=3	0.011407
8 month	74.2% N=3	0.035616	77.3% N=3	0.010612	75.0% N=3	0.0120
12 month	65.6% N=3	0.00241	62.98% N=3	0.023339	65.1% N=3	0.002921

Figure18 Follow up results of dexamethasone oral solutions assay from Elfashir, Khartoum and Stability cabinet

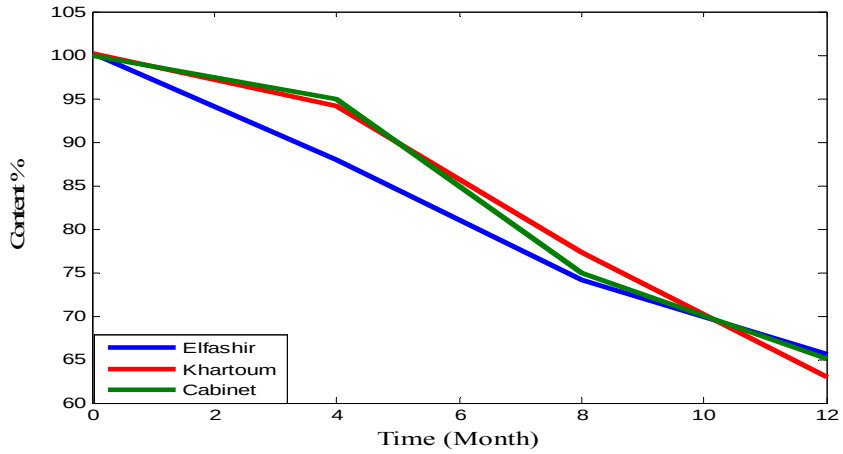


Figure 19 :

Comparison between assay results of dexamethasone oral solutions from different areas over 12month:

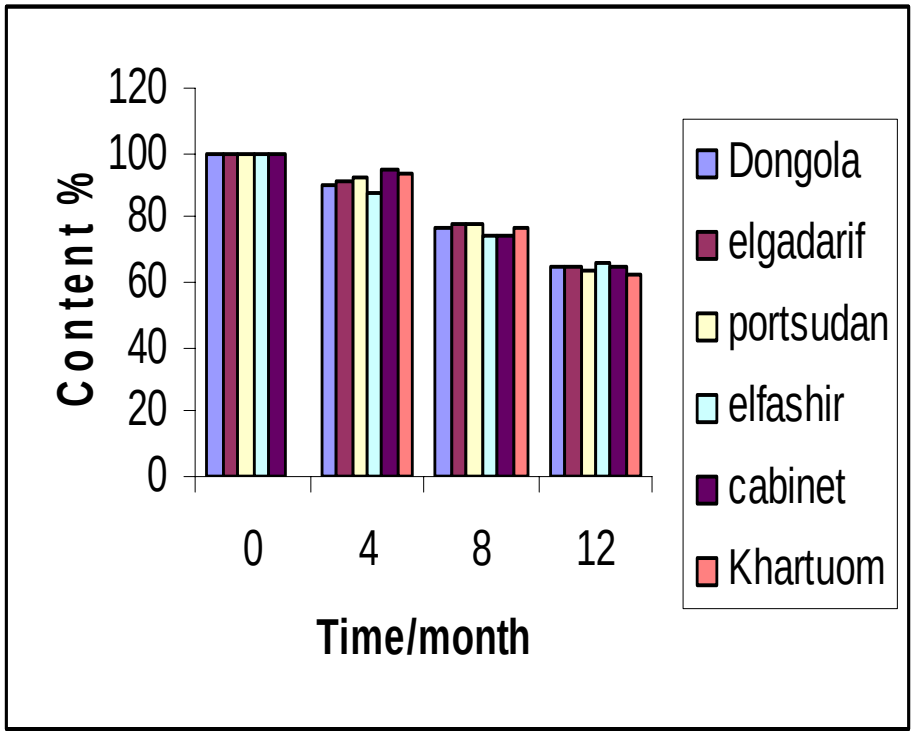
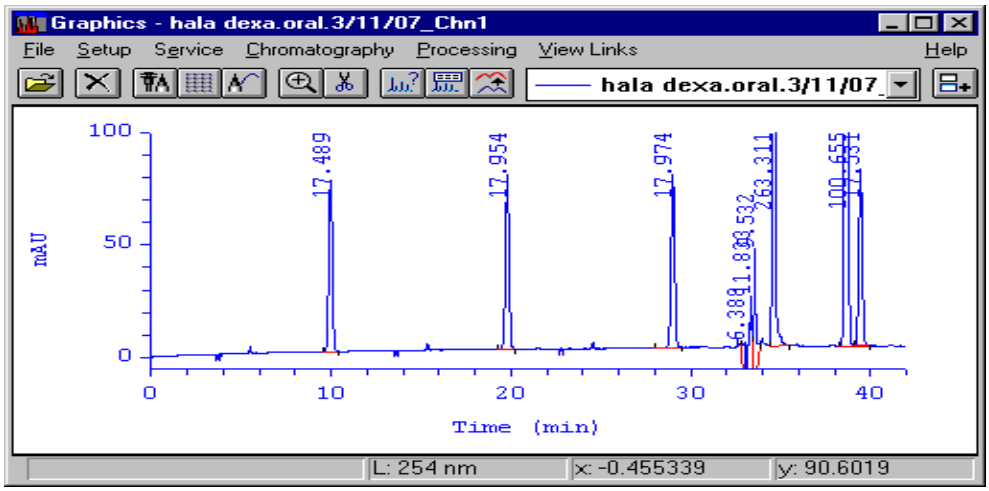


Figure 20: Examples of HPLC chromatogram for dexamethasone oral solution

- **HPLC chromatogram for initial assay**



- **HPLC chromatogram for assay after 8 month from Khartoum**

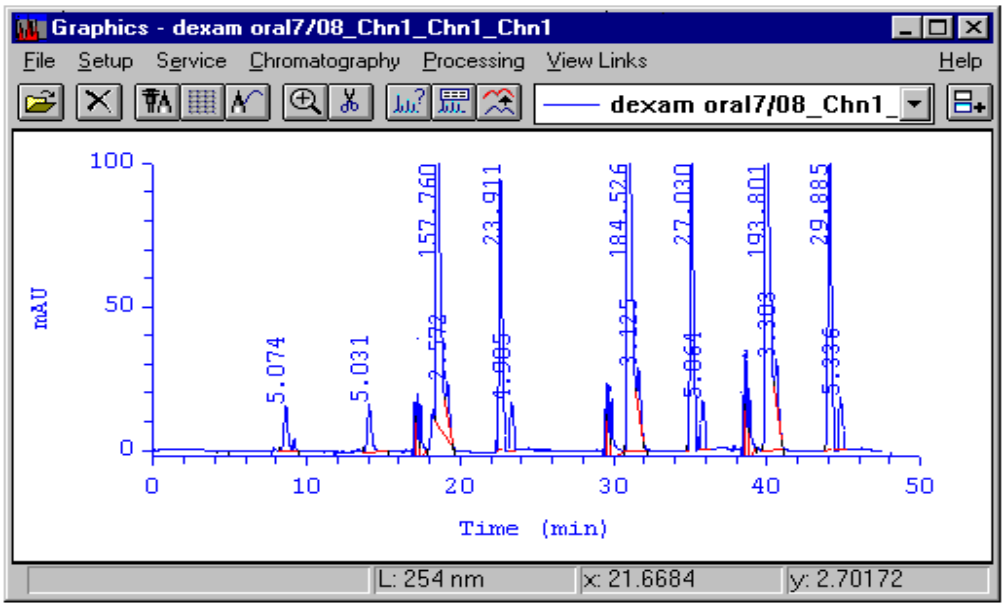


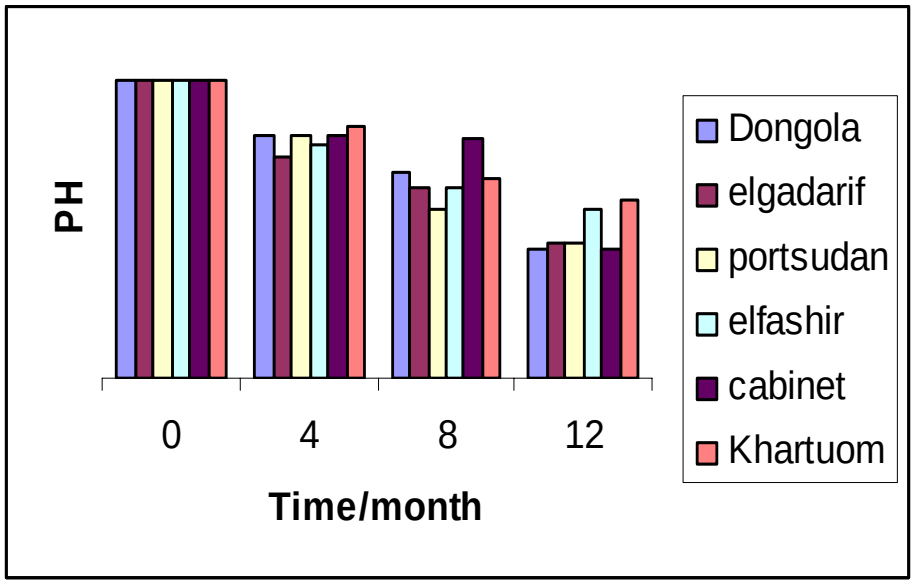
Table24 :

Follow up results for dexamethasone oral solutions PH:

	Dongola	Elgadarif	Port-Sudan	Elfashir	Khartoum	Stability cabinet
Zero time	3.1	3.1	3.1	3.1	3.1	3.1
4 months	2.97	2.92	2.97	2.95	2.99	2.97
8 months	2.88	2.85	2.8	2.85	2.87	2.96
12months	2.7	2.72	2.79	2.8	2.82	2.70

Figure 21.

Comparison between PH results of dexamethasone oral solutions from different areas over 12month



4.5. Results of proposed method validations:-

4.5.1. Sensitivity:-

Figure 22.HPLC chromatogram for dexamethasone tablets (zero time) added to dissolution media and directly injected to HPLC system at 240 nm

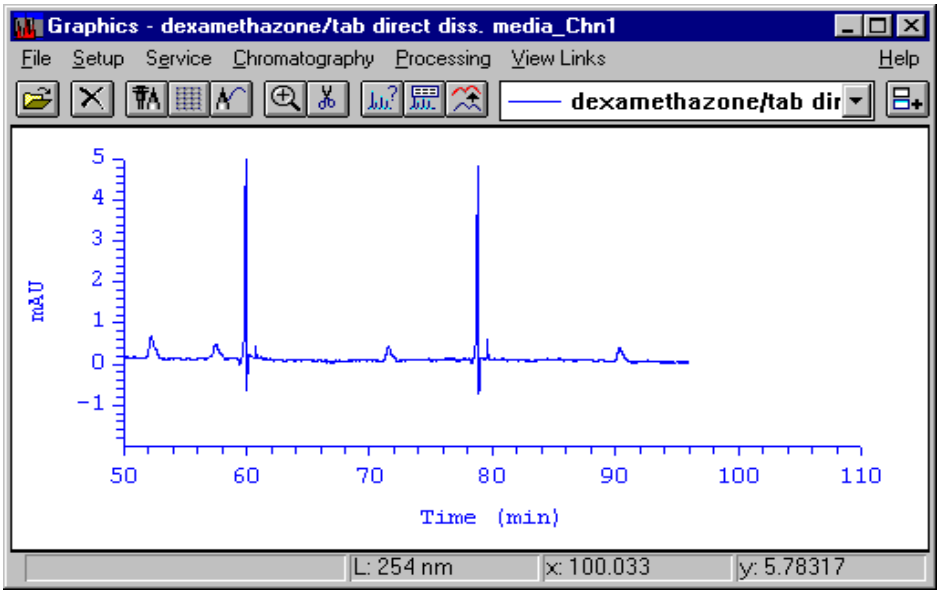
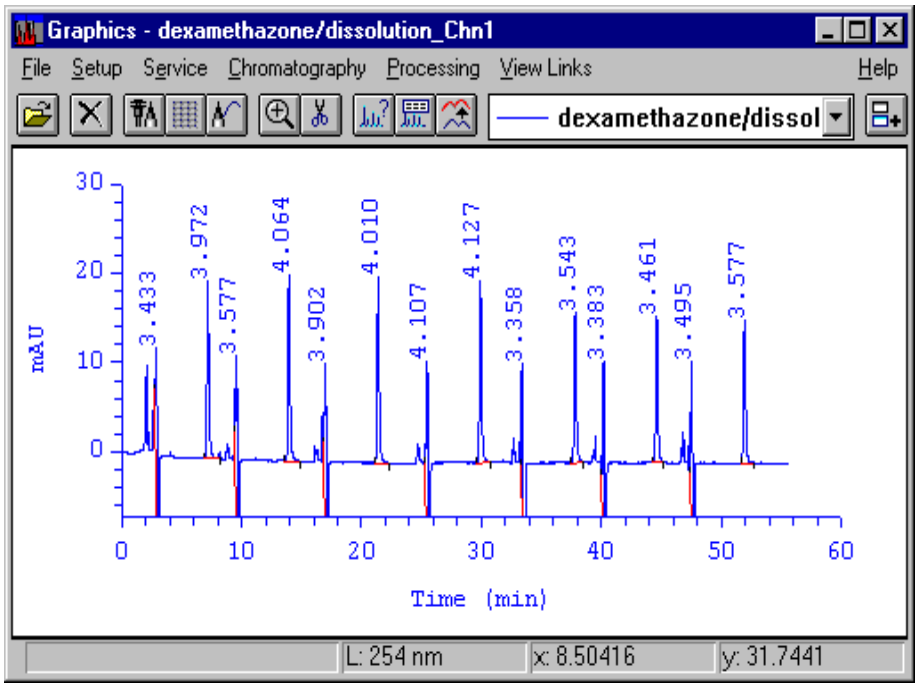


Figure23.

HPLC chromatogram for dexamethasone tablets (zero time) added to dissolution media and injected to HPLC system at 240 nm after extraction process:



Result of dexamethasone dissolution media injected to HPLC system after treatment = 95.8 %

Table 25

show Comparison between ultraviolet absorbance of dexamethasone in dissolution media at 254 nm and 240 nm

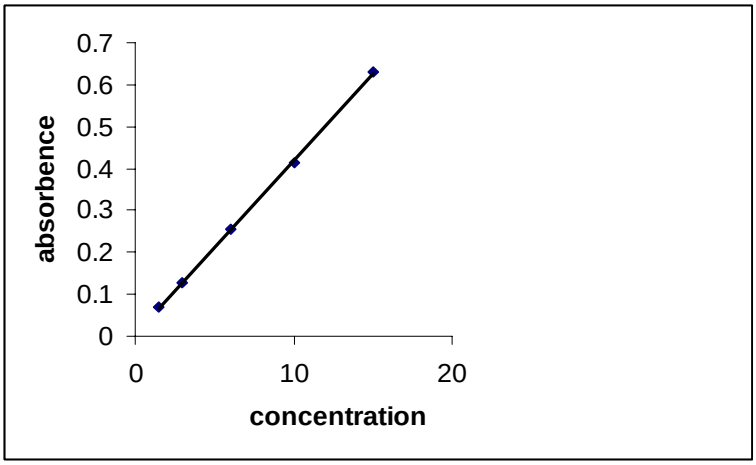
No	Absorbance at 240		Absorbance at 254	
	Standard	**sample**	**Standard**	**sample**
1	0.125	0.134	0.09	0.100
2	0.127	0.137	0.09	0.098
3	0.124	0.133	0.091	0.101
average	0.125	0.134	0.090	0.099
%content	107.7%		110.7%	

4.5.2. Linearity :-

Table 26 show ultraviolet absorbance for different concentrations of dexamethasone tablets after dissolution treatment using extraction method

concentration	absorbance	Content %
15.0 µg/ml	0.630	109.0
10.0 µg /ml	0.415	107.7
6.0 µg /ml	0.255	110.3
3.0 µg /ml	0.126	109.1
1.5 µg /ml	0.07	121.2

Figure 24. show linearity of the ultraviolet absorbance method for dissolution using extraction method



Y=0.0415X+0.0044

R^2 =0.9998

4.5.3. Limit of quantitation :-

Table No 27 show the absorbance of different concentrations of dexamethasone tablets after dissolution treatment using extraction method

concentration	absorbance
15.0 µg/ml	0.630
6.0 µg/ml	0.255
3 .0µg/ml	0.126
1.5 µg/ml	0.07

4.5.4. Accuracy:-

Table 28 Comparison between actual and theoretical results of Ultraviolet absorbance for different concentrations of dexamethasone tablets after dissolution treatment

No	Concentrations	Theoretical		Actual	
		Absorbance	Content%	Absorbance	Content%
1	3.6 µg/ml	0.138	110%	0.134	108.6%
2	3.3 µg/ml	0.127	100%	0.126	99.17%
3	3.0 µg/ml	0.115	90%	0.111	87.3%

4.5.5. Selectivity:-

Table 29 Comparison between absorption wave length of dexamethasone, Prednisolone and clobetasone (have structural similarity)

Sample	Absorbance wave length	Absorbance
dexamethasone	243.8 nm	0.522
Prednisolone	247.8 nm	1.370
clobetasone	238 nm	1.587

4.6. Order of Reaction

4.6.1.Dexamethasone ointment :

Table No 30 Result of dexamethasone ointment assay from stability cabinet

Time/month	Content%	Log content %
0 month	110.6	2.043
4 month	110.0	2.041
8 month	107.4	2.031
12 month	104.5	2.019

Figure25For zero order (plot content %against time/month)

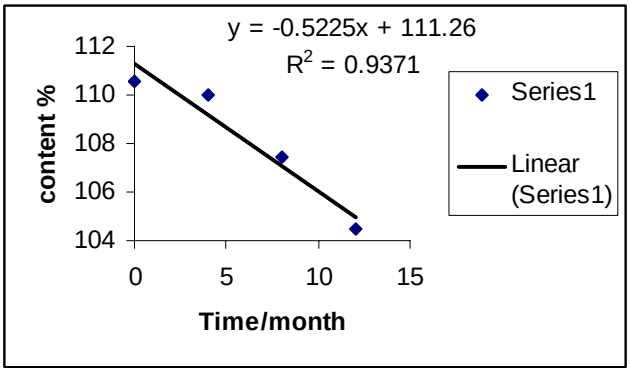
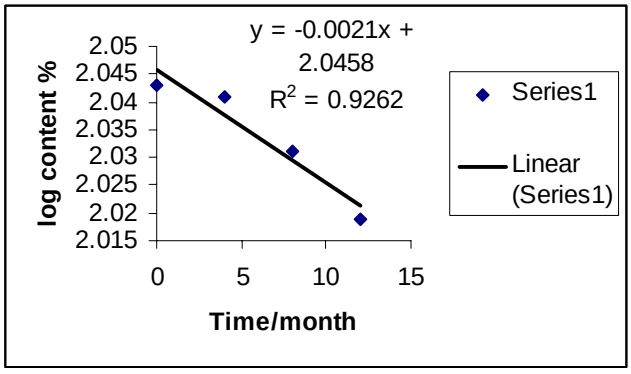


Figure 4.5.5For first order:

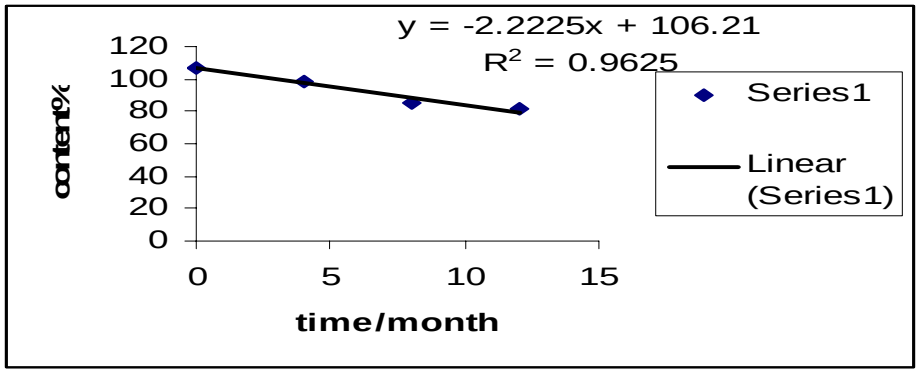


4.6.2. Order of reaction for Dexamethasone injection

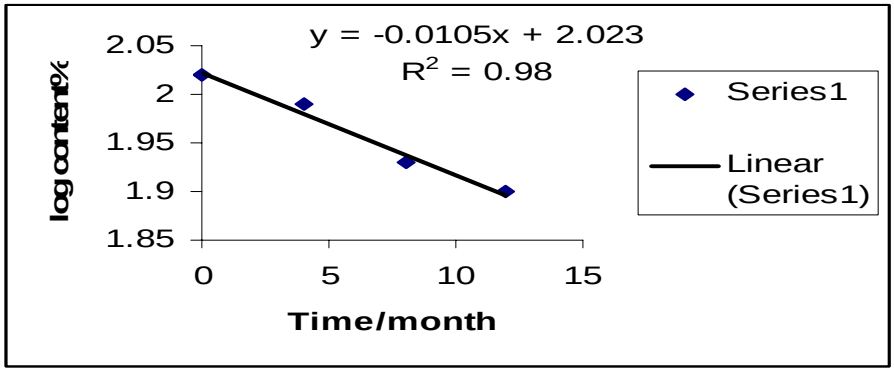
Table No 31 results of Dexamethasone sodium phosphate injections From Dongola:

Time/month	Content%	Log content %
0 month	106.3	2.026
4 month	98.8	1.99
8 month	85.2	1.93
12 month	81.2	1.90

Figure4.6.2.For zero order(plot content %against time/month)



For first order(plot log content %against time/month)



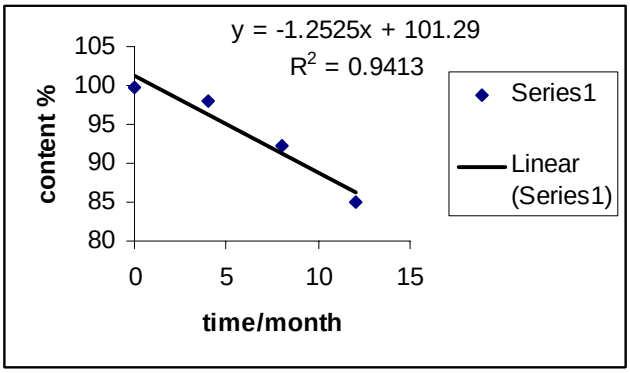
4.6.3. Order of reaction for Dexamethasone tablets

Table No 32.Result of dexamethasone (tablets) from Elfashir:

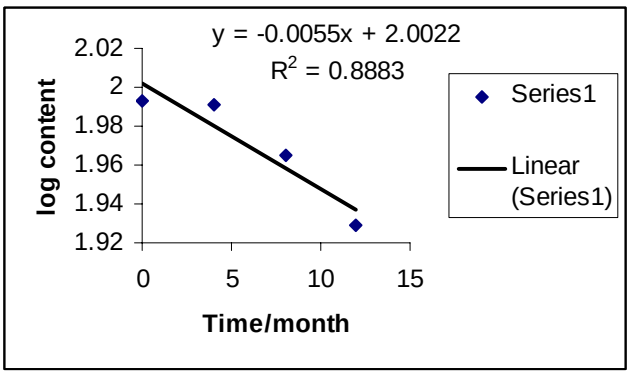
Time/month	Content%	Log content %
0 month	99.8	1.993
4 month	98.0	1.991
8 month	92.3	1.965
12 month	85.0	1.929

Figure 4.6.3.Order of reaction for dexamethasone tablets

For zero order(plot content %against time/month)



For first order (plot log content %against time/month)

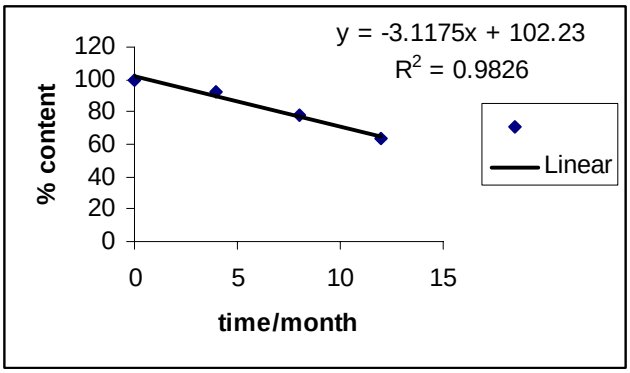


4.6.4. Order of reaction for Dexamethasone oral solution

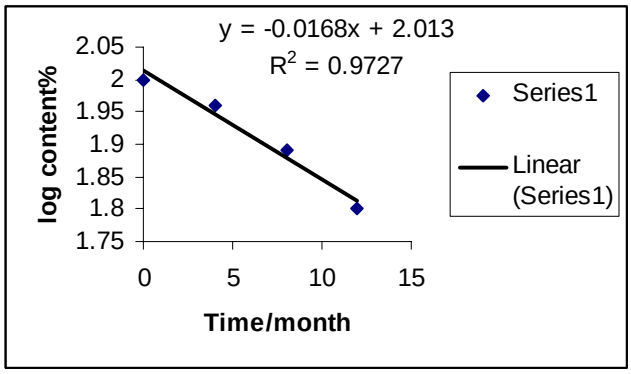
Table No 33 Result of dexamethasone Oral solutions from port Sudan:

Time/month	%content	Log content%
Zero time	100.2	2.00
4 month	92.5	1.96
8month	77.9	1.89
12 month	63.5	1.80

.Figure 4.6.4. For zero order (plot content %against time/month)



For first order (plot log content %against time/month)



Chapter
(6)
Discussion

Dexamethasone is a life saving drug used as a potent anti-inflammatory and anti-allergic.In common with all glucocorticoid their suppressive action on the hypothalamic-pituitary-adrenal axis is greatest and most prolonged when they are given at night; This is the basis of the 'over night dexamethasone suppression test' for diagnosing Cushing's syndrome. Dexamethasone is also appropriate for conditions where water retention would be a disadvantage. A corticosteroid may be used in the management of raised intracranial pressure or cerebral edema that occurs as a result of malignancy.It was found in different dosage forms in Sudan (tablets, injections, oral solutions and ointments) which are stable in normal controlled room temperature but when they were exposed to drastic conditions they may show changes due to instability. (British national formulary 57)

Dexamethasone usually follows oxidation reaction as degradation path way. (Walter Lund 12 edition)

According to the USP zone classification Sudan was classified as zone iii (hot/dry) in which the measured temperature in open air is 24.4 ºC and 39% R.H. .The WHO classify Sudan as zoon iii and iv in which the measured temperature at open air is 26.4 ºC and the RH is 77%; but the data obtained from the Metrological Authority show large fluctuations in temperatures during different seasons of the year and between day and night for the same area; for example:-

In Khartoum during January the temperature recorded at day is 30.7 ºC and at night is 15.6 ºC, but during May the temperature recorded at day is 41.9 ºC and at night is 27.6 ºC.

(**Metrological authority data sheet 2006)**

At the same time the majority of drug sealers (55%) have no transportation facilities and the majority of stores facilities are suffer from inadequate electrical supply; so care we must be taken about storage and transportation of pharmaceutical products in Sudan.

(Hussein A. H. august 2006)

Dexamethasone sodium phosphate injection should be protected from light and dexamethasone tablets should be stored in well-closed containers.

5.1. Results:-

The results of the present work for the studied dexamethasone different formulations were evaluated from the physical and chemical point of view.

5.1.1.Injections:-

The samples show little change in pH and appearance of the solution.

The chemical analysis of the samples at zero time (seven month after manufacture date)gave results of 106.3% ±(0.0127) table (9).After 8 month of distribution it showed 20% - 25% deteriorations in different areas of Sudan, compared to samples in the stability cabinet which showed 15% ±(0.00096) table (10)deteriorations this mean that the samples in pharmacies were exposed to storage conditions exceeded that found in the stability cabinet (30±2C˚and 65%RH).After one year all the samples showed 20%-30% deteriorations ,when these samples were compared with that one which was stored in well controlled conditions (C.M.S.) which the content % dropped from 106.3% to 103.12% after one year ,this can be considered as a reasonable change , this comparison indicate that the storage conditions in pharmacies play the main role in the observed deteriorations.

5.1.2. Oral solutions:-

The samples showed little change in pH and physical appearance of the solution.

The chemical analysis of the samples at zero time (5 month after manufacture date) 100.2% ±(0.0084) table (23).After 4 month of distribution it showed 6% - 13% deteriorations in different areas of Sudan, compared to samples in the stability cabinet showed 5% ±(0.0114)(table 24)deteriorations, this mean that the samples in pharmacies were exposed to storage conditions exceeded that found in the stability cabinet (30±2C˚and 65%RH). After one year all the samples showed 35%-37% deterioration, when these samples were compared with that one which was stored in well controlled conditions (company store)after one year it's content % dropped from 100.2% to 94.5% after one year ,this can be considered as a reasonable change , this comparison indicate that the storage conditions in pharmacies play the main role in the observed deteriorations.

5.1.3. Tablets:-

The samples showed an increase in dissolution test reading after 4 month(tables 15 - 20) which could be due to presence of the degradation product which absorb at the same wave length of the drug (the method is not selective).

The chemical analysis of the samples at zero time (11 month after manufacture date) 99.8 % ±(0.0162) table (12 and13).After 8 month of distribution it showed 5% - 9% drop in different areas of Sudan included samples stored in the stability cabinet. After one year all the samples showed 7% - 13% drop**.** This mean the effect of storage condition in the tablets is less than that of injections**.**

Comparison between tablets (from another company) stored in pharmacy and company stores were results the following; after 6 month both samples were showed about 14% drop, this means that the company store was not have adequate storage conditions(we use samples from other company because our samples which stored in the company store were missed) .

5.1.4. Ointments:-

The samples showed no change in its physical state and appearance.

The chemical analysis of the samples at zero time (2Years after manufacture date) 110.6 %±(0.0151) table (21 and 22).After 12 months of distribution it show 1% - 10% drop in different areas of Sudan include samples stored in stability cabinet ,This mean the effect of storage condition in the ointment is less than that of oral solutions, injections and tablets.

From all of this we can say:-

1. The different dosage forms of dexamethasone (representing the drug substance) which stored in the real storage conditions in the market (pharmacies and ware houses) especially oral liquids, injections and tablets were become out of the USP limits long time before their expiry dates. This means that the circulating drugs are becoming of low qualities and possibility of toxic decomposition products that might lead to use of non effective and non safe drugs .

2. The deterioration of some dosage forms stored in the stability cabinet may be due to:-

2.1. The electrical supply disconnection from the cabinet during may/2008 for 15 days.

2.2. The drug formula was not stable in storage conditions used for on going stability testing which recommended for registration.

3. The dosage forms had very fast deterioration in their concentrations in the period between May and august (hottest seasons); this indicates that the storage conditions were not well controlled and monitored.

5.2. Kinetic studies of dexamethasone stabilities:

The stability study for different formulations of dexamethasone revealed a first order reaction figures (41, 42,43and44).

The calculated rate of reaction, t_{10} and t_{50} were:

- For dexamethasone sodium phosphate injection:

K =0.693/ = 0.3118

t_{10} =0.105/0.3118 = 0.3367 month

t_{50} =0.693/0.3118 =2.2225 month

For dexamethasone tablets:

K =0.693 / 0.0055 =126

t_{10} =0.105/126 =- 0.00083 month

t_{50} =0.693/126 =-0.00 55 month

For dexamethasone ointments:

K =0.693 /0.0021 =330

t_{10} =0.105/ 330 = -0.00031month

t_{50} =0.693/330 =- 0.00 21month

For dexamethasone oral solutions:

K =0.693 / 0.0168 = 41.25

t_{10} =0.105/41.25 =-0.00254 month

t_{50} =0.693/41.25 =-0.0168month

5.3. Validation of the proposed ultraviolet method used for dissolution test for tablets:-

5.3.1Sensitivity:-

The UV method was found to be sensitive when the results compared with that obtained from the same solution using HPLC method, that the ultraviolet absorption value (0.678-0.648) while the area under the peak obtained from the HPLC system (3.35-3.495).

When the dissolution media was directly injected to the HPLC system the peaks obtained was so small and negligible.

The ultraviolet absorption for the dissolution media at 240 nm and 254 nm was found to be weak compared with that of treated dissolution media ,meanwhile the absorption value at 254 nm was found to be less than 0.1 .

5.3.2 Linearity:-

The UV method was found to have linear relationship between the concentration and absorbance with regression value (R^2) = 0.9998.

5.3.3Limit of quantitation:-

The minimum concentration that can be detected using this UV method is 0.000003 g/ml (3 μg/ml).

5.3.4 Selectivity:-

The UV method was found to be not selective, that it can not select the dexamethasone from other steroids and degradation products, so another method should be chosen .

5.3.5Accuracy:-

The UV method was found to be accurate as the standard deviation of the actual results from the theoretical results were not more than 2.7%**.**

Chapter (7)

Recommendations

1. The drug companies, stores and distribution facilities must be under strong control of the regulatory authority.

2. The pharmacies storage conditions must be obligatory monitored and recorded and they have to leave the conditioning system acting all over the day (24 hours) or to keep drugs in the refrigerator.

3. The regulatory authority must take strong punishments against any company gave unreal data about the stability of their drugs.

4. Massive drug stability studies should be done on most of drugs that circulate in Sudan especially in areas out of Khartoum providence.

(8)

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